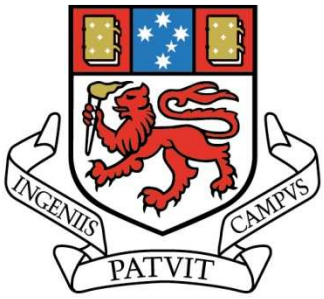

UNIVERSITY
OF TASMANIA

Select high value Australian finfish: Residues and contaminants of importance to public health and market access

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Doctor of Philosophy

JULY 2011

DECLARATION OF ORIGINALITY

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STATEMENT OF CO-AUTHORSHIP

The following people and institutions contributed to the publication of the work undertaken as part of this thesis.

I certify that the published manuscripts from this thesis are the product of my own work. I was responsible for performing all of the experiments, collecting all the data in the field and laboratory using techniques described in the thesis, analysing the data, producing the draft manuscripts, managing all changes in response to the co-author's recommendations and managing the manuscripts through the review and publication process. In accomplishing my research goals I did receive assistance and I hereby acknowledge the contribution of other researchers to my published manuscripts.

Paper one (Chapter two)

Padula, D.J.; Madigan, T.L.; Kiermeier, A.; Daughtry, B.J.; Pointon, A.M. 2004. Levels of dioxins (PCDD/PCDFs) and polychlorinated biphenyls (PCBs) in a random sample of Australian aquaculture-produced Southern Bluefin Tuna (*Thunnus maccoyii*). Organohalog. Compounds 66, 2074-2079.

Details of the authors' roles

Mr Thomas L. Madigan from the South Australian Research and Development Institute (Adelaide, Australia) offered general sample preparation assistance.

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Dr Andrew Pointon from the South Australian Research and Development Institute (Adelaide, Australia) assisted with the project idea, its formalisation, development and intellectual support.

Paper two (Chapter three)

Padula, D.J.; Daughtry, B.J.; Nowak, B.F. 2008. Dioxins, PCBs, metals, metalloids, pesticides and antimicrobial residues in wild and farmed Australian Southern Bluefin Tuna (*Thunnus maccoyii*). Chemosphere 72, 34-44.

Details of the authors' roles

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Prof. Barbara F. Nowak from the University of Tasmania (Launceston, Australia) provided intellectual support and technical expertise.

Paper three (Chapter four)

Padula, D.J.; Madigan, T.L.; Nowak, B.F. Australian farmed Yellowtail kingfish (*Seriola lalandi*) and Mulloway (*Argyrosomus hololepidotus*): Residues of metallic, agricultural and veterinary chemicals, dioxins and polychlorinated biphenyls. Accepted for publication in Chemosphere.

Details of the authors' roles

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Prof. Barbara F. Nowak from the University of Tasmania (Launceston, Australia) provided intellectual support and technical expertise.

Paper four (Chapter five)

Padula, D.J.; Daughtry, B.J.; Flint, R.; Nowak, B.F. Mercury content of wild Australian Southern Bluefin Tuna (*Thunnus maccoyii*) destined for aquaculture fattening: Comparison with bluefin tunas in the Japanese market. In preparation.

Details of the authors' roles

Mr Ben J. Daughtry from Food Standards Australia New Zealand (formerly South Australian Research and Development Institute) (Canberra, Australia formerly Adelaide, Australia) offered support with field collection activities, general sample preparation, project planning and assistance with data presentation.

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Paper five (Chapter six)

Padula, D.J.; Daughtry, B.J.; Flint, R.; Nowak, B.F. Mercury content of Australian canned Skipjack Tuna (*Katsuwonus pelamis*) products. In preparation.

Details of the authors' roles

Mr Ben J. Daughtry from Food Standards Australia New Zealand (formerly South Australian Research and Development Institute) (Canberra, Australia formerly Adelaide, Australia) offered support with general sample preparation, statistical analysis, project planning and assistance with data presentation.

Ms. Rose Flint from the South Australian Research and Development Institute (Adelaide, Australia) assisted with statistical analysis and data presentation.

Prof. Barbara F. Nowak from the University of Tasmania (Launceston, Australia) provided intellectual support and technical expertise.

Paper six (Chapter seven)

Padula, D.J.; Nowak, B.F. Causes of detentions and rejections of Australian seafood in international trade. In preparation.

Details of the authors' roles

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University of Tasmania	**University of Tasmania**

Date: _____

ACKNOWLEDGMENTS

A great number of people have provided support and assistance during my period of candidature who I would like to personally thank. There are many others in addition to those I named individually below. To each of you named and unnamed please accept my heartfelt thanks and gratitude.

Mr Brian Jeffriess (Australian Southern Bluefin Tuna Industry Association) foremost has been an inalienable source of encouragement, support and wisdom during my study. Through all the difficulties I encountered you always provided confirmation of the need to finish my study.

My supervisor Prof. Barbara F. Nowak (University of Tasmania) who I enjoyed many manuscript review meetings over raspberry waffles at Christmas Hills farm and Huon Valley mushrooms at the Black Cow during my visits to Launceston. Your guidance helped shape my creative spirit and fostered my ability to write as a scientist.

My family, who have long endured during their visits my kitchen table piled high with notes and reference materials plus my fellow students who I shared my thoughts and musings with over pots of Russian Caravan tea at Stillwater, Ritchies' Mill. Also, my friends in Adelaide who I enjoyed nights out with at My Yen and afternoon teas around my kitchen table over scones accompanied by raspberry jam and clotted cream.

I would lastly like to thank my employer; the South Australian Research and Development Institute for allowing me to complete my study and in doing so allow the development of scientific capability to address the future needs of the Australian seafood industry.

"I can't change the direction of the wind, but I can adjust my sails to always reach my destination."

James Byron Dean (1931-1955)

ABSTRACT

Consumers worldwide expect access to safe seafood products regardless of its origin. Each country judges imported products against its own residue and contaminant standards coupled with differing testing capability, sample collection, sample preparation and data reporting strategies. Australian exporters of finfish whether it is farmed or wild capture origin have to negotiate their way through a series of regulatory hurdles and gate keeper organisations in each market they wish to target. Detention or destruction of product at border control stations can occur if importing country standards are not met. This thesis outlines five separate case studies of how this has been successfully done with select high value Australian finfish species by tackling different issues of public health and market access concern.

The General Introduction provides an overview of the researched finfish species and introduces the key residues and contaminants addressed in this thesis.

Chapter two introduces dioxins and PCBs data for single season grow-out farmed Australian Southern Bluefin Tuna (*Thunnus maccoyii*) SBT. The mean concentration of dioxins found was 0.53 pg WHO TEQ/g (range 0.2-1.1) while the mean concentration of PCBs found was 32 μg/kg (range 13-55).

Chapter three more comprehensively addresses single season grow-out farmed Australian SBT not just for dioxins and PCBs, but pesticides, antibiotics and metallic elements. Japanese port of entry official sampling methods are discussed. The mean concentration of dioxins in wild SBT was 0.14 pg TEQ/g (range 0.07-0.32) and in farmed SBT was 0.2 pg TEQ/g (range 0.06-0.8). The mean concentration of the sum of dioxins and dioxin-like PCBs in wild SBT was 0.27 pg TEQ/g (range 0.18-0.45) and in farmed SBT was 1 pg TEQ/g (range 0.2-4). Mean PCB concentration in wild SBT was 0.13 pg TEQ/g (range 0.1-0.21) and in farmed SBT was 0.8 pg TEQ/g (range 0.17-3.5). Mean PCB total concentration in wild SBT was 0.47 μg/kg (range 0.4-0.6) and in farmed SBT was 6.6 μg/kg (range 0.8-41). The mean concentration of total mercury in wild SBT was 0.34 mg/kg (range 0.28-0.42) and in farmed SBT was 0.31 mg/kg (range 0.18-0.45). There were no detectable levels of any pesticide or antimicrobial compounds in any sample of SBT.

Chapter four introduces farmed Yellowtail Kingfish (*Seriola lalandi*) YTKF and Mulloway (*Argyrosomus hololepidotus)*. Data on dioxins, PCBs, pesticides, veterinary medicines and metallic elements are presented and discussed in terms of Australia, Japanese and European regulatory standards. The mean concentration of dioxins in YTKF was 0.6 pg TEQ/g (range 0.22-0.8) and in Mulloway was 0.16 pg TEQ/g (range 0.16-0.16). The mean concentration of dioxins and dioxin-like PCBs in the YTKF was 2.6 pg TEQ/g (range 1.4-3.5), while the Mulloway had a mean concentration of 0.67 pg TEQ/g (range 0.57-0.76). The mean concentration of PCBs in YTKF was 21 μg/kg (range 8.6-29) and in Mulloway was 5.4 μg/kg (mean 4.7-6). The mean concentration

of dioxin-like PCBs in YTKF was 2.1 pg TEQ/g (range 1.2-2.8) and in Mulloway was 0.51 pg TEQ/g (Range 0.41-0.61). The mean mercury concentration in YTKF was 0.03 mg/kg (range 0.02-0.05) and in Mulloway it was 0.023 mg/kg (range 0.02-0.04). There were no detectable levels of any pesticide or antimicrobial compounds in any sample of YTKF or Mulloway.

Chapter five addresses total mercury content in wild Australian SBT. Results are discussed in terms of international regulatory standards and compared with bluefin tunas available in the Japanese market. Mean total mercury concentration was 0.43 mg/kg (range 0.24-0.72). A dietary exposure assessment found that pregnant women and women planning pregnancy could consume one 150 g serving per week, the rest of Australian general adult population could consume up to three 150 g servings per week and children (up to 6 years) could consume one 75 g serving of Australian wild SBT per week.

Chapter six investigates total mercury content of canned wild Skipjack Tuna (*Katsuwonus pelamis*) products. The mean total mercury content found in 2005 was 0.10 mg/kg (range 0.07-0.15) and in 2006 was 0.09 mg/kg (range 0.02-0.22). Testing of condiment canning ingredients identified low levels of mercury in black pepper and cinnamon. A dietary exposure assessment found that pregnant women and women planning pregnancy could consume seven 150 g servings per week, while the rest of the Australian adult general population could consume up to 14 150 g servings per week and children (up to 6 years) could consume eight 75 g servings of these canned tuna products per

week. The use of fixed body weight values in exposure assessments may bias results when compared to actual current body weight values of the Australian population. These issues are discussed in conjunction with the inability of consumers to readily identify low mercury content tuna species from back of packaging labelling.

Chapter seven describes the causes of detentions and rejections of Australian seafood products in international trade. On a numerically basis, cadmium in Australian crustaceans is ranked the highest notification category. However, caution is urged when assessing these lists without access to qualifying information on frequency of testing, number of tests undertaken, sampling methodology, laboratory analytical techniques and reporting basis. Standards in each notification instance are compared between the importing country and those in Australia. Fish names are discussed and their application to identification of product in trade is highlighted.

The General Discussion summarises outcomes of research covered in this thesis and identifies future product integrity research needs and priorities for the Australian seafood industry to meet current and future market access needs.

ACRONYMS

ERL:	Extraneous Residue Limit
Hg:	Mercury
LOD:	Limit of Detection
LOQ:	Limit of Quantification
LOR:	Limit of Reporting
MAL:	Maximum Allowable Level
ML:	Maximum Level
MRL:	Maximum Residue Limit
PCBs:	Polychlorinated biphenyls
PCDD:	Polychlorinated dibenzo-*p*-dioxin
PCDF:	Polychlorinated dibenzofuran
SA:	South Australia
SBT:	Southern Bluefin Tuna (*Thunnus maccoyii*)
TEF:	Toxic Equivalency Factor
TEQ:	Toxic Equivalent
YTKF:	Yellowtail Kingfish (*Seriola lalandi*)
WHO:	World Health Organisation

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CHAPTER ONE

General Introduction

During the last few decades our knowledge on residues and contaminants in foods of marine origin has increased due to several factors including advances in analytical capability, publication of public health occurrence studies and greater international trade of seafood (Díaz-Cruz and Barceló a, 2007; Herzog, 1970). In parallel, an increase in capture and production of fisheries products has occurred providing a source of income and livelihood to many people (Figure 1). Domestically and internationally regulatory standards have been established in most developed countries or regions specifying acceptable levels of chemical hazards for fisheries products in trade (McWilliam, 1991; Berg and Licht, 2002; Wilson et al, 2003, Lugard and Smart, 2006). Each country or region manages its own internal domestic food regulatory standards, sometimes with reference to international standards set by Codex Alimentarius Commission (Horwitz, 1982; McMillan, 1991; Malich et al, 1998; Renwick et al, 2003). Fish products in international trade violating importing countries' standards (when judged by the importing country's port of entry authority) may be detained or destroyed (Ababouch et al, 2005; Ababouch, 2006; Valdimarsson, 2004). Negative public perceptions about seafood have been created following discovery of a range of potentially harmful chemical contaminants in wild and farmed fish species; recent research is identifying and confirming the positive public health benefits of regular fish consumption (Tanabe, 1988; Redshaw, 1995; Zhang et al., 1999; Jacobs et al., 2002; Hites et al., 2004; Mozaffarian and Rimm, 2006; Domingo, 2007a; Domingo, 2007b; Domingo, 2007c; Martı´-Cid et al., 2007; Kelly et al., 2008; Sapkota et al., 2008; Kleter et al., 2009).

Australia harvests a wide variety of high value farmed and wild capture finfish; currently little data exist for these Australian finfish species to underpin international market access or support public consumption advisories. Internationally agreed risk analysis protocols to allow assessment of public health risks in food are being applied to seafood products (Hathaway, 1993; Renwick et al, 2003). The European Union (EU) has taken a leading role in this process (Bergeaud-Blackler and Ferretti, 2006).

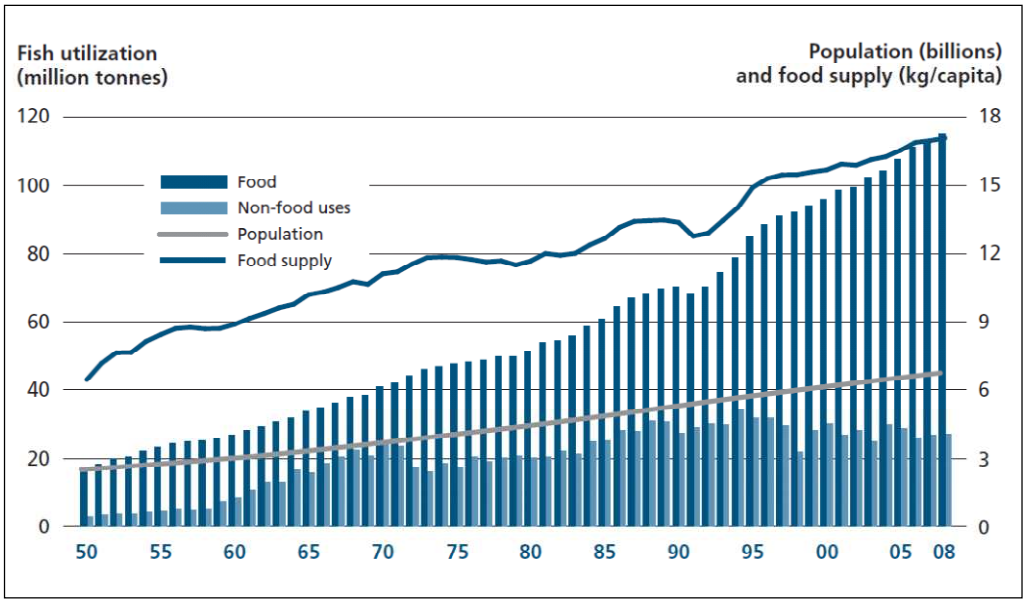

Figure 1. World fish utilisation and supply during 1950 to 2008. (Pulvenis de Séligny et al, 2010).

Australian international seafood markets

Australia's seafood industry in 2009/2010 generated a gross total income of $2.2 B (Pham, 2010). Of that $1.4 B was attributable to wild capture seafood products (Pham, 2010). Over this period $1.5 B of Australia's total seafood products was exported (equates to 68% by value) (Pham, 2010).

In 2009/2010 Hong Kong was Australia's biggest importer by value of Australian seafood products (Pham, 2010). The emergence of affluent Chinese populations desiring Western luxury seafood products has seen the growing dominance of Chinese speaking countries as major purchasers of Australian seafood products (Pham, 2010). However, the development of these countries' food regulatory systems has not always kept pace with consumer expectations (Wilcock et al, 2004).

Dioxins and polychlorinated biphenyls (PCBs)

Dioxins are a group of 75 polychlorinated dibenzo-*p*-dioxin (PCDD) and 135 polychlorinated dibenzofuran (PCDF) congeners of which 17 are of confirmed public health concern (Mitrou et al, 2001; Schecter et al, 2006). The most toxic congener is 2,3,7,8-tetrachlordibenzo-*p*-dioxin (TCDD) against which all other congeners are rated (Van den Berg et al, 1998; Van den Berg et al, 2005). Dioxins (PCDD/PCDFs) are not intentionally manufactured, rather they are a by-product of many industrial processes: high temperature garbage incineration, agro-chemical manufacture, paper bleaching and in natural processes such as bushfires (Schecter, 2006). The uptake and transfer of these pollutants through the marine food chain to fish and ultimately to humans is incremental over an extended period of time (Niimi, 1996; Wang et al, 1998; Nowak, 1991a).

Polychlorinated biphenyls (PCBs) belong to a group of 209 different chlorine-substituted congeners; various trade names exist for these technical mixtures (Schulte and Malisch, 1983; Appendices 1, 2 and 3).

Historically PCBs were used as cooling oils in electrical power transformers due to their stability and thermal properties (Ross, 2004). Twelve of these congeners have similar chemical properties to PCDD/PCDFs and are known as dioxin-like PCBs (Van den Berg et al, 1998).

The toxicity of dioxins and PCBs to human health through their endocrine disrupting effects has been demonstrated in uncontrolled accidental releases such as the BASF phenoxy herbicide plant in Germany in 1953; Seveso phenol chemical plant explosion in Italy in 1976; Yusho rice oil poisoning in Japan in 1968 and the Yucheng cooking oil poisoning in Taiwan in 1979 (Zober et al, 1998; Piacitelli, 2000; Mocarelli, 2001; Aoki, 2001; Cole et al, 2003). Fatal outcomes were recorded in some instances from these events (immediately, or over a period of time); other affected population groups (including their children) suffered permanent medical problems following exposure.

Standards for PCBs differ markedly in international markets (Martin et al, 2003). In Australia, compliance testing for PCBs is undertaken using Aroclor 1254 and Aroclor 1260 during national surveys (Department of Agriculture, Fisheries and Forestry, 2011). In Japan total PCBs have been assessed in some fish species using technical mixtures of Kaneclor 300, Kaneclor 400, Kaneclor 500 and Kaneclor 600 (Ueno et al, 2003). In the EU standards for PCBs are set based on congener weighted toxicity values, Toxic Equivalence Factors (TEFs) (European Commission, 2006; Appendix 4).

Dioxins and PCBs have long half-lives measured in years or decades in the natural environment (Tanabe, 1988; Geyer et al, 2002; Schecter, 2006). Dioxins and PCBs tend to bio-accumulate in fatty tissues of fish due to being extremely hydrophobic, resistant to degradation, thermally stable and having a very high lipid affinity (Tanabe, 1988). This lipophilic property is a problem for aquaculture fish, as high-energy lipid diets are generally fed to captive fish (Biswas et al, 2009). Uptake of these pollutants from aquaculture feed in species such as Atlantic Salmon (*Salmon Salar*) is variable but can be predicted based on known levels in feeds (Berntssen et al, 2007).

Mercury

Mercury is a harmful metallic element that occurs in several chemical forms; the most toxic form is the methylated form of mercury (Peterson et al, 1973). It is principally released by natural processes in the marine environment such as deep sea volcanic vents (Peterson et al, 1973). Its chemical properties allow it to accumulate more rapidly in large top predatory species in the marine environment; intervention strategies may be applied in aquaculture fish such as bluefin tunas (Peterson et al, 1973; Nakao et al, 2009; Ando, 2010). Public health campaigns have until recently adopted a warning strategy to consumers not to eat certain types of fish (such as top predatory species like shark) which many consumers have simply interpreted as to avoid eating any fish (Shimshack and Ward, 2010).

Regulatory standards for mercury in seafood have been informed and shaped by large epidemiological studies in the Faroe Islands of fish

eating populations (Shipp et al, 2000). Accidental mercury poisonings such as occurred in Basra, Iraq and Minamata, Japan have also provided information on epidemiology of acute tainted food exposure (Clarkson et al, 1976; Weiss, 2007). In Japan fish consumption accounts for approximately 80-90% of the total human mercury exposure (Zhang et al, 2009).

Pesticides

Organochlorine compounds such as DDT may persist in the marine environment and occur in fish of economic importance such as bluefin tunas for decades after terrestrial usage due to their ability to accumulate in lipids (Kannan et al, 1994; Ruus et al, 2002; Ueno et al, 2002). Some pesticide compounds may have application in aquaculture systems for control of aquatic weeds in ponds or for treatment of some parasite infections (Burridge et al, 2010). Due to their environmental stability some pesticides may persist for many years following historical application even after product registration has ceased (Connell et al., 2002).

Some of Australia's former agricultural chemicals manufacturing sites have left measurable contamination in the marine environment such as in Sydney, New South Wales (Rudge et al, 2008, Food Authority of New South Wales, 2011). Experimental agriculture in new regions such as in the Ord River area of Western Australia has left farmland contaminated with pesticides such as DDT, aldrin and dieldrin applied to control unwanted insect pests on new crop species (Fredericks and Palmer, 2008). Crop protection chemicals such as endosulfan applied to

cotton crops have been found in wild fish inadvertently contaminated through seasonal run-off (Nowak and Julli, 1991b).

Veterinary medicines

Administration of veterinary medicines such as antibiotics or anthelmintics to aquaculture stock is sometimes necessary to manage animal welfare issues. Residues of these compounds may not be fully removed from treated fish due to retention of the parent compound or its metabolites in medicated fish (Sapkota et al, 2008; Burridge et al, 2010).

Residues of veterinary medicines in aquaculture fish can occur following administration to treat aquatic diseases and the withholding period not being fully observed (affected by variable water temperature), but may in some cases arise from inadvertent point sources to the marine environment, such as sewage treatment plant effluent (Katae et al, 1980; Hirsch et al, 1999; Kenkyo, 2000). Wild fish can also inadvertently accumulate residues of veterinary medicines from treatment of caged aquaculture fish in the same waters via bathing or medicated feed (Björklund et al, 1990).

Sampling methods for compliance purposes

Each market has adopted its own frontier border inspection systems for conformance assessment with domestic standards. These systems may or may not be internally harmonised between different ports of entry and may or may not share aspects with major trading partners.

Of key interest to Australian exporters of Southern Bluefin Tuna (SBT) is the port of entry sample collection procedures of the Japanese Ministry of Agriculture, Forestry and Fisheries (MAFF) who are responsible for testing of imported SBT (Ministry of Agriculture, Forestry and Fisheries, 2011; Appendix 5). MAFF have developed a cross carcase composite sampling approach based on collecting samples from 6 defined portions from 10 individual tunas (of the same species, origin and import batch) (pers. comm. Mr Yuichi Nagaka. 2004. Japanese Ministry of Agriculture, Forestry and Fisheries; Appendix 5).

In contrast the Japanese Ministry of Health, Welfare and Labour (MHLW) has a different sampling approach to assess compliance with regulatory standards set within the *Food Sanitation Law* and within the *Positive List System*. MHLW's approach is to sample filleted portions of tunas immediately after the auction process at the buyer or processor's premises at the Tsujiki Fish Market (or local market), Tokyo (it is considered that at this point the tuna goes from being an agricultural product to being a consumer ready food) (pers. comm. Mr Kenji Doi, MHLW). The Japanese food regulatory system is described in more detail in Appendix 6. In contrast, other countries take different approaches to sample collection and portion to which standards apply to (Appendix 7).

The Australian Government Australian Quarantine and Inspection Service takes samples of imported seafood for compliance purposes following the Australian Government Attorney General's Department Imported Food Control Regulations 1993 under Schedule 1 Selection of

Samples (regulation 22) (Attorney General's Department, 1993). This standard prescribes appointed analysts and sample numbers to be collected for official analyses. The Regulations include random sampling of imported product, risk food heightened surveillance and risk food reduced surveillance categories. Based on the number of units in the lot, set sample numbers are prescribed which must be taken for testing. This could see as few as two or as many as 126 sample units taken for testing depending on the category of testing which the imported product falls into (Attorney General's Department, 1993). Documentation checks and exporting country of origin competent authority attestations are assessed in addition to testing.

International bodies such as the Codex Alimentarius Commission have established a range of technical committees to develop standards for international product in trade; methods of sampling are part of this remit (Appendix 8). Many countries including Australia are members of Codex; adoption of Codex standards by individual member countries may be complete or selective depending on existing domestic standards and other competing priorities.

Species studied

Each of the four Australian marine finfish species covered in this thesis is illustrated below with brief notes following describing its diet, culinary use and markets.

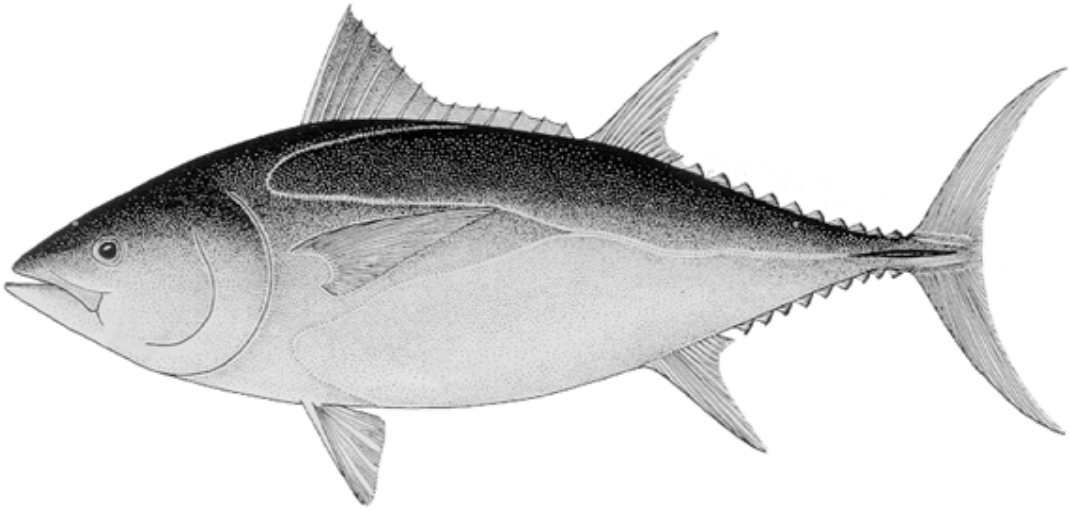

Figure 2. Line drawing of Southern Bluefin Tuna (*Thunnus maccoyii* - Castelnau, 1872) (Froese and Pauly, 2011).

Wild Southern Bluefin Tuna (*Thunnus maccoyii*) (SBT) feed on a wide variety of marine fishes, crustaceans, cephalopods, salps, and other marine aquatic animals (Young et al, 1997; Froese and Pauly, 2011). SBT are highly prized in Japan in sushi and sashimi dishes for its rich oily meat. Reproduction is age dependent in this internationally managed fast growing tuna species (Thorogood, 1986; Bergin and Haward, 1994; Carter, 1998).

Aquaculture production of SBT is centred on Port Lincoln, SA in the Spencer Gulf region (Primary Industries and Resources South Australia, 2011). Almost all of Australia's total production is exported to Japan as whole chilled or frozen product (Pham, 2010). Some product is also exported to Europe, China and Hong Kong, South Korea and to the United Arab Emirates (Pham, 2010).

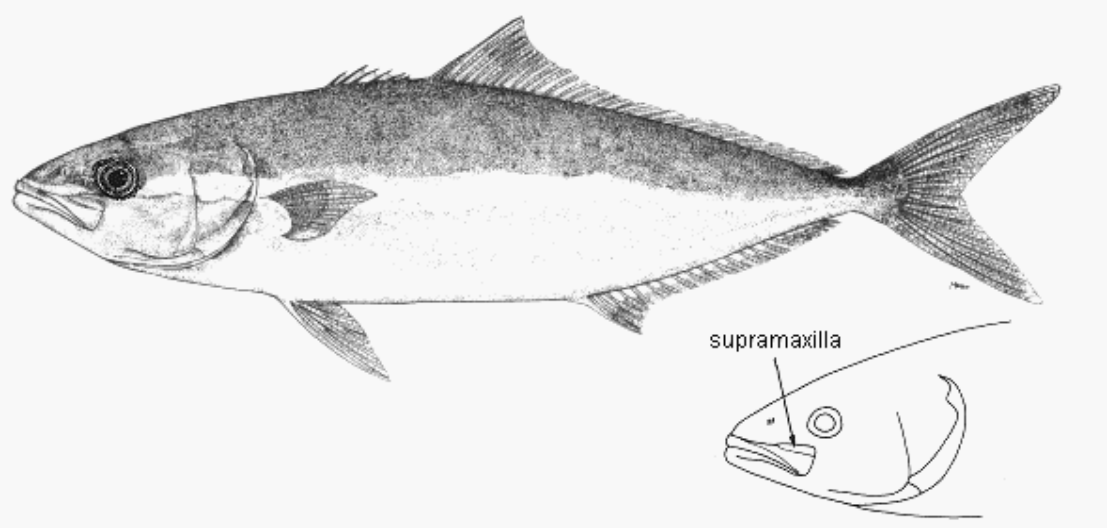

Figure 3. Line drawing of Yellowtail kingfish (*Seriola lalandi* - Valenciennes, 1833) (Froese and Pauly, 2011).

Wild Yellowtail Kingfish (*Seriola lalandi*) (YTKF) feed on small marine fishes, squid and crustaceans (Froese and Pauly, 2011). It is generally eaten cooked but premium fish are used for sushi and sashimi dishes (Froese and Pauly, 2011). Processed fish are exported to a range of markets including Europe, Japan, China, Hong Kong and the United States (Pham, 2010).

Aquaculture production is centred at several sites including Arno Bay, SA, Whyalla, SA and Port August, SA in the Spencer Gulf Region (Primary Industries and Resources South Australia, 2011)

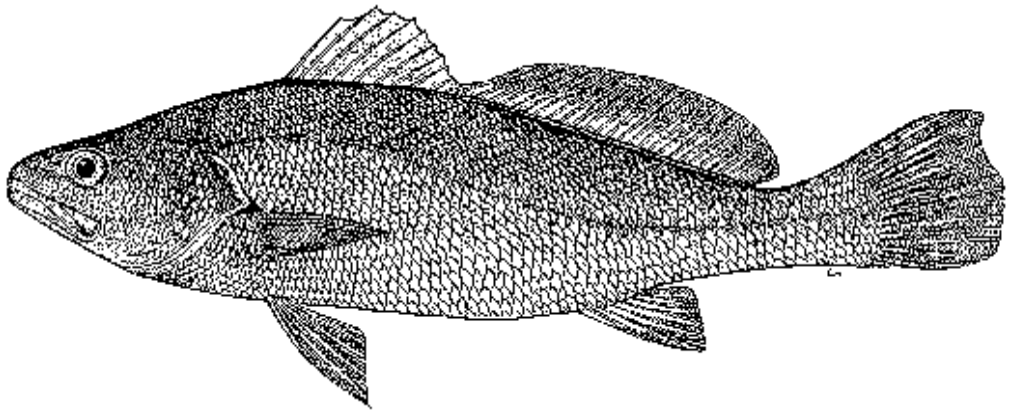

Figure 4. Line drawing of Mulloway (*Argyrosomus hololepidotus* - Lacépède, 1802) (Froese and Pauly, 2011).

Wild Mulloway feed on cuttlefish, crabs, prawns, and marine worms (Froese and Pauly, 2011). It is generally eaten cooked but premium fish may be used for sushi and sashimi dishes (Froese and Pauly, 2011). Aquaculture product is exported to the EU in addition to being sold on the Australian domestic market (Pham, 2010).

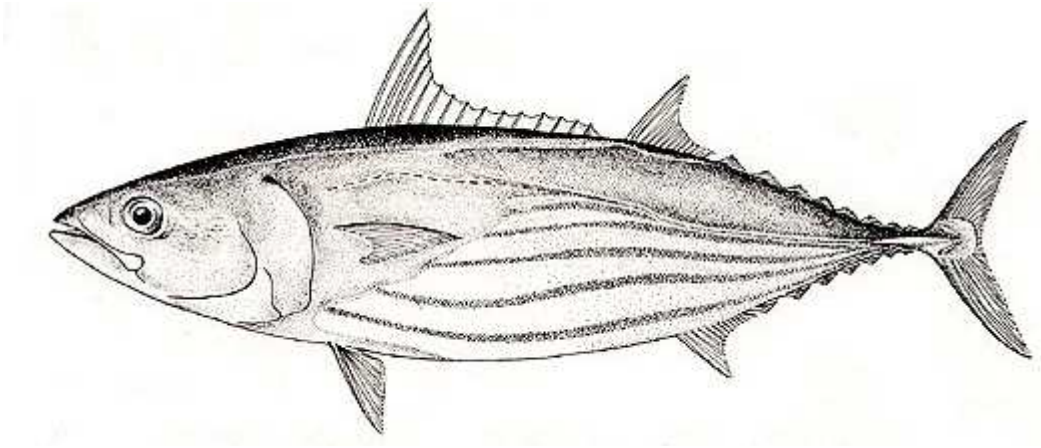

Figure 5. Line drawing of Skipjack Tuna (*Katsuwonus pelamis* - Linnaeus, 1758) (Froese and Pauly, 2011).

Wild Skipjack Tuna (*Katsuwonus pelamis*) feed on marine fishes, crustaceans, cephalopods and molluscs (Froese and Pauly, 2011). Its mild taste makes it amenable to blending with flavour ingredients to make pre-prepared meal products. In Australia this species is utilised predominantly for canning (Froese and Pauly, 2011).

Aims and thesis outline

1. To benchmark residues and contaminants of importance to public health and market access using methodology of port of entry inspection authorities to inform risk assessment evaluations of select high value Australian marine finfish species.
2. To assess results against Australian domestic and international food regulatory standards.
3. Examine the causes of rejections and detentions of Australian seafood products in international markets.

The following thesis chapters present results from six different case studies examining high value Australian finfish. The current publication status (published, submitted, in preparation) of each chapter is presented on the title page. Each of the published manuscripts has been edited in a uniform consistent style to be accommodated in the thesis.

Chapter two is the first Australian publication of dioxins and PCBs data for single season grow-out aquaculture-produced Australian SBT. It summarises results from a pilot study which later led to the case

study which forms Chapter three. The study helped the Australian Government identify sampling strategies which were incorporated into the National Dioxins Program study design (these form Chapter three).

Chapter three more comprehensively addresses the data gaps not just for dioxins and PCBs, but pesticides, antibiotics and metallic elements including mercury in single season grow-out farmed SBT. An independent economic evaluation estimated the value of this residue and contaminant profile to the SBT industry at $150 M per annum in relation to market access (Agtrans, 2008). Similar economic evaluation has been carried out for YTKF and Mulloway research (Agtrans, 2010). Further work has now led to access to the Chinese market in addition to the EU. Chinese authorities were informed by the study when developing their own standard SC/T 3117-2006 - Tunas for raw consumption (Standards Press of China, 2006).

Chapter four addresses two new aquaculture species, YTKF and Mulloway. The study presents baseline residue and contaminant data for a wide range of compounds of trade significance in these two species of marine finfish. Results from this study led to access to the EU market for these two species and formed the basis for the current EU residue control program for aquaculture products (Feazey, 2011).

Chapter five investigates total mercury content of wild Australian SBT that are destined for aquaculture fattening and export to Japan. A dietary exposure assessment was performed which produced information on the number of servings of wild SBT that Australian

consumers could eat. Differences in exposure assessment methods used by Japan and Australia are discussed in terms of use of fixed body weight values. Regulatory standards for mercury in tuna are presented for a number of different countries. Japanese Government port of entry total mercury test results for the period 2002-2004 are presented for comparison, along with Japanese wild catch and farmed tuna species results.

Chapter six examines Australian canned Skipjack Tuna and its total mercury content. The study characterises the total mercury content of these canned products and finds a few surprising condiment ingredients with measurable mercury content. A dietary exposure assessment demonstrates these products are a low risk to consumers. Comparative total mercury results with canned tuna products available in New Zealand and the United States. Mercury standards that apply to canned tuna products internationally are summarised. Inconsistent naming of tuna species combined with non-disclosure of tuna canning species on product labels is discussed in relation to transparency to consumers wishing to select low mercury content canned tuna products.

Chapter seven examines the cause of detentions and rejections of Australian seafood in international trade over the period 2000-2011. Results are presented against importing country vs. Australian food regulatory standards. Results are discussed and compared with international causes in other markets and against Australian port of entry testing for imported seafood products. Discussion includes an

emphasis that caution should be used when interpreting numerical lists of international notifications without access to qualifying information on frequency of testing, non-disclosed notifications, number of shipments inspected, sampling information, laboratory methodology and importing country's standards technical basis.

Chapter eight is a general discussion of the findings of this thesis including recommendations for future research and policy development for the Australian seafood industry.

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CHAPTER THREE

Published in

Padula, D.J., Daughtry, D.J., Nowak B.F. 2008. Dioxins, PCBs, metals, metalloids, pesticides and antimicrobial residues in wild and farmed Australian Southern Bluefin Tuna (*Thunnus maccoyii*). Chemosphere 72, 34-44.

1. Introduction

Aquaculture has allowed the sustainable production of food-producing marine finfish in many countries. With this new production system, product integrity risks have been identified that may alter public perception and confidence in the safety of aquaculture-produced marine finfish. The presence of residues of agricultural chemicals and environmental contaminants in foods of aquatic animal origin has led to greater calls for information on the presence of these compounds in products entering overseas markets at frontier border inspection stations (European Food Safety Authority, 2005). Since the initial discovery of PCBs in fish in 1966, greater regulatory controls have been placed on the presence of these and other related halogenated xenobiotic residues in seafood products entering overseas markets (Anonymous, 1966). Ambiguity in market access requirements related to inconsistent adoption of international phytosanitary measures may impede international trade of seafood. Seafood still is an important source of essential minerals necessary for human health such as iodine, selenium and zinc in addition to essential fatty acids and fat-soluble vitamins (Horrocks and Yeo, 1999, Dahl *et al*., 2006).

Southern Bluefin Tuna (SBT) are a migratory species of pelagic marine finfish that occupy Australian and international waters. Breeding takes place in tropical Indian Ocean waters off Java between September and April. Juvenile SBT then migrate down the coast of Western Australia. From December to April schools of juvenile SBT, two-four years of age, congregate in surface waters off Southern Australia particularly in the Great Australian Bight off the coast of South Australia (SA). Older SBT,

greater than five years are only occasionally found in near-shore Australian surface waters along the Australian Continental Shelf. Wild caught SBT for farming in Port Lincoln are caught by the purse seine method in the Great Australian Bight. The introduction of sea-cage capture-based aquaculture for SBT occurred in Port Lincoln in 1991 (Aiken et al., 2006). The major market for Australian SBT is Japan.

The accumulation of residues such as PCDD/PCDFs, PCBs and mercury reflects trophic status in the marine food chain (Anders et al., 2002). There are in total 210 PCDD/PCDF congeners of which 17 are of regulatory significance and a further 12 dioxin-like PCBs with similar regulatory status. The dioxin-like PCBs (non-ortho and mono-ortho substituted congeners) are extremely thermally stable and resistant to degradation by photolytic and microbial processes. Unlike PCBs, PCDD/PCDFs were never commercially produced, but are by-products of industrial processes including the manufacture of pesticides, high temperature waste incineration and the bleaching of paper products. Chlorinated contaminants such as PCDD/PCDFs and PCBs are persistent in the marine environment due to their persistence in fat (Tanabe, 1988). These compounds are only sparingly soluble in water due to their Henry's constant (Bamford et al., 2000). The source and fate of these compounds varies from country to country (Arisawa et al., 2003). Some of these compounds have confirmed public health risks (Mitrou et al., 2001). As ubiquitous environmental contaminants in foods of animal origin, the main source of PCDD/PCDFs and PCBs in the human diet is from fatty foods of animal origin (Charnley and Doull, 2005).

Increasingly around the world, measures are being considered to introduce source-directed methods to reduce dietary-related exposure to these contaminants in fatty foods of animal origin, including aquaculture fish. The Australian Competent Authority, Food Standards Australia New Zealand (FSANZ) guiding principle for the protection of public health and safety is modelled on the As Low as Reasonably Achievable (ALARA) principle (Abbott et al., 2003).

Other environmental contaminants such as pesticides may be present in fish due to agriculture and coastal run-off. Some crop protection chemicals such as DDT may be persistent in the marine environment due to historical terrestrial usage, relative stability in the marine environment and resistance to degradation. Antimicrobial residues may arise when prescribed veterinary medicine withholding periods are not observed before harvesting. Metals such as mercury are present in all seafood at varying concentrations due to natural sources such as volcanoes and deep-sea geothermal vents but may also arise from other terrestrial activities.

The Japanese Ministry of Agriculture, Forestry and Fisheries (MAFF) undertakes testing of imported wild and cultured bluefin tunas from countries such as Australia for PCDD/PCDFs and PCBs. The Japanese Ministry of Health, Labour and Welfare (MHLW) has responsibility for risk management functions such as setting of Maximum Residue Limits (MRLs) and verification of compliance with domestic regulatory standards. The Japanese Food Safety Commission (FSC) has

responsibility for undertaking risk assessments for MHLW such as methylmercury in seafood.

In this study farmed SBT were fed a mixture of Australian and United States of America wild caught baitfish diets. The dominant species of baitfish used in 2004 were Australian sardines, *Sardinops neopilchardus,* United States sardines, *Sardinops sagax* and Australian redbait, *Emmelichthys nitidus nitidus.* A minor component of mixed origin baitfish species such as squid and anchovies were also fed to the SBT during the 2004 season. In the wild, SBT are opportunistic feeders, eating mainly cephalopods (squid and octopi), crustaceans and baitfish such as sardines.

The aim of this research was to benchmark the concentrations of a range of natural and environmental residues and contaminants of market access importance in wild and farmed Australian SBT. Part of this study was undertaken for the National Dioxins Program within the Australian Government Department of Environment and Heritage (DEH) in conjunction with the Australian Government Department of Agriculture Fisheries and Forestry (DAFF).

Study area

The township of Port Lincoln is located within the Spencer Gulf region of South Australia (SA) (Figure 1). This region has no major rivers or large population centres around the catchment area, hence there are few terrestrial sources of pollution entering the Spencer Gulf.

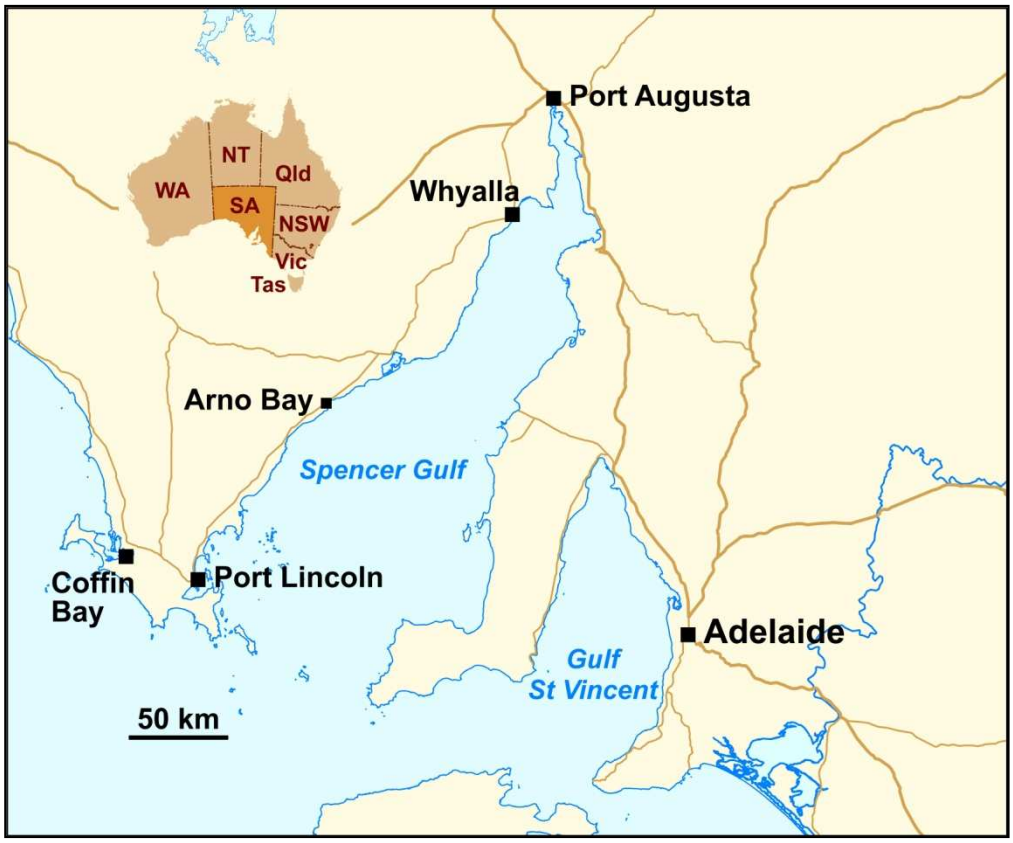

Figure 1. Map of the Spencer Gulf region of South Australia showing Port Lincoln, home of Australia's Southern Bluefin Tuna farming.

2. Materials and Methods

2.1. Sample collection

Wild SBT (n=5) were obtained from a commercial tow-in in April 2004. Farmed SBT (n=26) were collected from all 12 Australian producers of farmed SBT. Commercial tuna divers manually caught each individual SBT during commercial harvesting operations during May to June 2004. Thus, the fish provided were representative of fish commercially harvested for export to overseas markets (Burger et al., 2006). The whole SBT carcase(s) was collected (following gilling and gutting) from each company. The number of fish collected from each individual company reflected the pro-rata production basis of that establishment

to the total production of Australian farmed SBT in 2004. The mean fork-length was 105 cm (range 96-108) for wild SBT and 102 cm (range 76-127) for farmed SBT. The mean weight (gilled and gutted) of wild SBT was 20 kg (range 14-23) and 24 kg (range 11-45) for farmed SBT.

2.2. Sample processing

The Japanese Ministry of Agriculture, Forestry and Fisheries (MAFF) sample imported bluefin tunas following a schedule based on taking selected portions from 10 separate bluefin tunas to form a composite sample for testing (Figure 2). The approach taken in this study was an abridged version of this method, in that the composite sample was formed from an individual SBT carcase not from 10 separate SBT. The reason for doing this was to compare residue results for individual fish. All bones, skin and dark muscle tissue were removed so that only the edible portion was included in the samples to be analysed. Samples were homogenised in a stainless steel HOBART™ food processor and stored at -40°C. Between processing each SBT all benches, tools, food processor implements, knives etc were cleaned with DECON-90 laboratory detergent and double rinsed with mains tap water. Samples for PCDD/PCDF and PCB analyses were wrapped in aluminium foil to prevent photo-degradation changes in sample integrity and packed inside two plastic bags to prevent sample contamination. Sample identification integrity was protected through placing the label on the inner of the two bags to avoid any freezer-associated label losses.

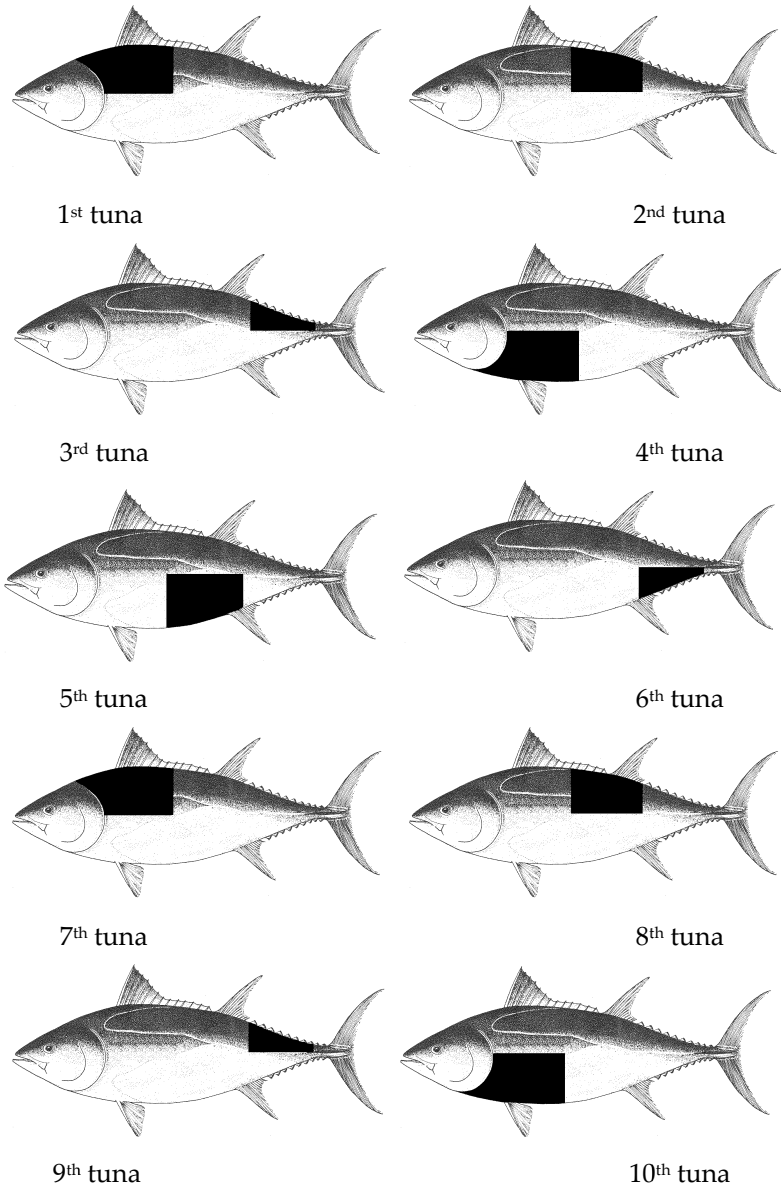

Figure 2 Diagrammatic representation of the Japanese Ministry of Agriculture, Forestry and Fisheries (MAFF) sample collection protocol for bluefin tunas based on the portions indicated (in black) from 10 different bluefin tunas. These 10 individual samples from each of the bluefin tunas are then pooled to form the analytical sample.

2.3. Chemical analyses

2.3.1 PCDD/PCDFs and PCBs

PCDD/PCDF and PCB analyses were undertaken by a New Zealand laboratory accredited by International Accreditation New Zealand (IANZ) to ISO/IEC 17025 (1999).

2.3.2 Sample Preparation

Samples (thawed at 4°C for 24 hours with all thawing juices included in the analytical sample) (40 g) were blended with powdered sodium sulfate (BDH, analytical reagent grade) and then loaded into a Soxhlet extractor, fortified with labeled internal standards (EPA-1613LCS and 68A-LCs, Wellington Laboratories) and extracted with organic solvent (methylene chloride:hexane 1:1) (Mallinckrodt Ultim, analytical reagent grade) for 16 hours. A clean-up and recovery standard was then added and the extract was evaporated to constant weight and the lipid weight determined gravimetrically. Clean-up was undertaken by solid phase (in-house prepared columns) clean-up techniques using acid and base modified silica gel (silica gel 60, Merck). The extract was purified with column chromatography techniques using activated alumina (aluminium oxide 90 active acidic, Merck) and carbon (carbopack C 60/80 mesh, Supelco). The final extract was concentrated and fortified with recovery standards (EPA-1613ISS and 68A-ISS, Wellington Laboratories) and analyzed by High Resolution Gas Chromatography - High Resolution Mass Spectroscopy (HRGC-HRMS).

2.3.3 PCDD/PCDFs and PCB determination

Analysis was carried out on a Micromass Autospec Ultima High Resolution Mass Spectrometer interfaced to an Agilent 6890 chromatograph operating in the splitless mode equipped with Zebron ZB-5 capillary columns for PCB and PCDD/PCDF. Analysis followed United States Environmental Protection Agency (USEPA) method 1613B for PCDD/PCDFs and USEPA method 1668A for PCBs. HRMS analysis was carried out in the electron impact mode. Native and labeled compounds were acquired by Selected Ion Monitoring (SIM) with the mass resolution being maintained at 10,000 (5% valley) throughout the analysis. Chromatographic data were processed using QuanLynx™ (Waters MassLynx™ software package).

Analysis for PCBs included the non-ortho PCB congeners: 77, 81, 126, 169, mono-ortho PCB congeners 105, 114, 118, 123, 156, 157, 167, 189. In addition, a group of indicator PCB congeners was measured: 1, 3, 4, 15, 19, 28, 37, 44, 49, 52, 54, 70, 74, 99, 101, 104, 110, 138, 153, 155, 170, 180, 183, 187, 188, 194, 196, 199, 202, 205, 206, 208 and 209. Concentrations of individual congeners were determined via isotope dilution. Total PCB concentration was determined by the summation of individual concentrations of all detected PCB congeners with non-detects treated as being equal to the Limit of Reporting (LOR).

2.3.4 Quality assurance and quality control

The isotope dilution technique involves determining the native analyte levels using the ratio of responses for the native and internal (isotopically labeled) standards. Hence the native analytes are self-

corrected for recovery. Results were reported on a fresh weight (f.w.) and lipid weight (l.w.) basis.

2.3.5 Calculation of PCDD/PCDF and dioxin-like PCB Toxic Equivalents (TEQs)

For PCDD/PCDFs and dioxin-like PCBs each congener concentration (upper bound basis) was first standardised to its 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) TEQ concentration (that is TCDD has a value of 1). This approach allows risk assessment studies to be undertaken. The TEQ is formally defined as (Van den Berg et al., 1998):

$$TEQ = \sum_{n1}[PCDD_i \times TEF_i] + \sum_{n2}[PCDF_i \times TEF_i] + \sum_{n3}[PCB_i \times TEF_i] \quad (1)$$

Where

- TEQ is Toxic Equivalent concentration;
- n1, n2 and n3 are the number of PCDD, PCDF and PCB congeners in the sample;
- $PCDD_i$, $PCDF_i$ and PCB_i are the concentration of PCDD, PCDF and PCB congener i; and
- $(TEF)_i$ is the human Toxic Equivalency Factor (1998 values used; Van den Berg et al., 1998) value of the specific congener.

2.4 Metallic determination

Metallic analyses (Cd, Cu, Pb, Hg, Zn, As and Se) were undertaken by an Australian laboratory accredited by the National Association of Testing Authorities (NATA) to the requirements of ISO/IEC 17025 (1999). An in-house method (NT2.46 Trace metals in food and biota)

was used for sample preparation. A subsample (about 1 g) was taken from the defrosted and homogenised sample (thawed at 4°C for 24 hours with all thawing juices included in the analytical sample). This was digested with concentrated nitric acid (Merck, redistilled analytical reagent grade) and hydrochloric acid (Merck, redistilled analytical reagent grade) for two hours in a 95-100°C water bath. Analysis was performed by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) on an Elan 6100 DRC (Perkin Elmer). Analysis was based on USEPA methods 6010, 6020 and AOAC Methods 986.15, 974.14 (16th Edition).

2.4.1 Quality assurance and quality control

For every 20 samples one blank, one duplicate, one blank spike, one sample matrix spike and one in-house prepared laboratory control sample (prawn tissue) were run by the contract laboratory. Laboratory in-house control and spiked samples (including spiked blanks) recoveries were all between 80-120 %. Duplicates all had a Relative Percentage Difference (RPD) <24 %. Blanks were all less than the Limit of Reporting (LOR). All results were corrected for recoveries where necessary. All recoveries met internal NATA method requirements.

2.5 Pesticide determination

Pesticide analyses were undertaken by an Australian laboratory accredited by the National Association of Testing Authorities (NATA) to the requirements of ISO/IEC 17025 (1999). Samples were thawed by the contract laboratory at 4°C for 24 hours with all thawing juices included in the analytical sample. Sample preparation was undertaken

in accordance with an in-house method (NR36 Analysis of multi residues by GC/MS organochlorines, organophosphates, PCBs, fungicides, herbicides, synthetic pyrethroids and carbates). The entire sample was first re-homogenised. A subsample (1 g) of the re-homogenised sample was mixed with anhydrous sodium sulphate (Ajax, Finechem) then extracted with hexane:acetone 60:40 (Merck, chromatography grade). The extract was cleaned up using Gel Permeation Chromatography (Gilson ASPEC) and the final extract analysed by Gas Chromatography/Mass Spectroscopy (GC/MS) (Hewlett Packard 5973) in scan mode with PTV large volume injector (Agilent 5973). Analytes were quantified using the internal standard method - US EPA 8270 internal standard mix (Supelco, cat 4-8972). Organochlorine analytes were determined by Gas Chromatography (GC) twin column Electron Capture Detector (ECD) (Agilent 6890). The GC/MS pesticide method was adapted from the Pesticides Analytical Manual (FDA, PAM Vol. 1, 3rd edition, 1996). The GC/ECD method followed US EPA 8081.

3.5.1 Quality assurance and quality control

The contract laboratory ran samples in batches of 20 which included a matrix blank, spike and duplicate analyses. The recoveries for spikes were all between 70 to 130%. Blanks were all less than the Limit of Reporting (LOR) for all batches. All results were corrected for recoveries. All recoveries met internal NATA method requirements.

2.5.2. Lipid content determination

Lipid content analysis was undertaken by an Australian laboratory accredited by the National Association of Testing Authorities (NATA) to the requirements of ISO/IEC 17025 (1999). Samples were thawed by the contract laboratory at 4°C for 24 hours with all thawing juices included in the analytical sample. A subsample (5 g) was weighed in a pre-dried soxhlet fat determination thimble. The thimble was placed in a soxhlet apparatus for at least 16 hours using diethyl ether (Merck, analytical reagent grade) on a constant temperature (90°C) water bath. The flask was dried in a convection oven at 102°C for two hours and then cooled in a desiccator to room temperature. The flask was returned to the oven and underwent successive re-weighings until no further weight loss occurred between successive dryings. The lipid content of the sample was calculated by subtracting the final flask weight from the initial flask weight and dividing this figure by the original sample mass. Results were then expressed as a percentage (%).

2.6 Antimicrobial determination - microbial inhibition test (MIT)

Antimicrobial analyses (beta-lactams, Cephalosporins, aminoglycosides, sulphonamides, tetracyclines, macrolides, lincosamdies) were undertaken by an Australian laboratory accredited by the National Association of Testing Authorities (NATA) to the requirements of ISO/IEC 17025 (1999). All results were corrected for recoveries.

2.6.1 Betalactams, macrolides/lincosamides, tetracyclines and aminoglycosides

Samples (thawed at 4°C for 24 hours with all thawing juices included in the analytical sample) (10 g) were first extracted by high-speed blender with a 1:1 mixture of acetonitrile (Merck, HPLC grade) and methanol (Merck, HPLC grade). The betalactams and macrolide groups are extracted into the solvent mix, whilst the tetracyclines and aminoglycosides bind to the tissue protein precipitate. The protein precipitate is extracted with EDTA (Sigma, analytical grade) solution and a dilute perchloric acid (Sigma, analytical grade) solution. The sample was then put through a C-18 and a SCX cartridge (Varian-Bond Elut) for clean up. This separated the tetracycline and aminoglycoside fractions. All fractions were then plated across multiple microbiological culture plates cultured with antimicrobial sensitive organisms. Cereus plate cultured with *Bacillus cereus*, Thermo plate cultured with *Geobacillus stearothermophilus*, Luteus plate cultured with *Bacillus luteus* and MIT B plate cultured with *Bacillus Subtillus*. MIT B plates were from Silliker Microtech. All other plates were prepared in-house.

Blank controls and spiked plates were run. For MIT screening, three different spikes were performed for each batch of samples. Each of these three spikes contained representative analytes for each group of antimicrobial compounds. Any detections by MIT were subjected to confirmatory method testing by HPLC and LCMS-MS with all analytes within each antimicrobial compound group spiked at LOR, 2xLOR and 5xLOR levels for each batch of samples.

2.6.2 Sulphonamides

Samples (thawed at 4°C for 24 hours with all thawing juices included in the analytical sample) (5g) were extracted using ethyl acetate (Ajax UNIVAR analytical reagent) in a high-speed blender. The ethyl acetate was then reduced by evaporation under nitrogen and then re-extracted in dilute hydrochloric acid (Merck, analytical grade). The sample was then derivatised with flourescamine before analysis by High Performance Liquid Chromatography (HPLC) (HP Angilent 1100) (reverse phase) with fluorescent detection. External standards (all obtained from Sigma Aldrich except Sulphatroxazole which was obtained from the Australian Government National Measurement Institute, Pymble Australia) were used for calibration (0.05, 0.10 and 0.2 mg/kg) and quantitation for all samples and quality control spikes.

2.7 Quality assurance and quality control

Duplicate samples were run every 10^{th} sample or a minimum of one duplicate per batch if <10 samples in the batch. All results were corrected for recoveries with recovery for each analyte required to fall within +/− 2 SD (Standard Deviation) of Running Mean (RM) recovery. RM recovery is based on an in-house control chart which records all recovery data including during method development and validation. All recoveries met internal NATA method requirements.

2.8 Data treatment and analyses

A linear mixed effect model was fitted to the data to investigate whether there was any difference in $\log_{10}$ PCDD/PCDFs & dioxin-like PCBs between lipid content, type ("Wild" or "Farmed") and the

interaction between lipid content and type. The producer was included in the model as a random effect. Assumptions were checked using standard diagnostic plots. An ANOVA was used to test for significant differences in $\log_{10}$ PCDD/PCDFs and dioxin-like PCBs between lipid content, type and the interaction between lipid content and type.

3. Results

The key factor driving differences between wild and farmed SBT for the fat-soluble residues and contaminants is the lipid content of the SBT itself. The highest mean fat content of 11.13 % (range 0.9-18) was found in the farmed SBT compared to 0.88 % (range 0.3-2.3) found in wild caught SBT (Table 3). This lipid content mirrors the lower PCDD/PCDFs and dioxin-like PCBs content of the wild SBT compared to farmed SBT (Figure 3, Table 3). The mean concentration of PCDD/PCDFs and dioxin-like PCBs were 0.97 pg TEQ/g (range 0.23-4.3) in farmed SBT, while in wild SBT the mean concentration was 0.27 pg TEQ/g (range 0.18-0.45) (Table 3). The variability between individual farmed SBT in terms of their PCDD/PCDF and dioxin-like PCB content can be attributed to feed strategies employed by individual producers, length of culture period, harvest time of year, age of tuna, sex of tuna and diet of wild SBT pre farming (Figure 3). The total PCB concentration was 0.47 ng/g (range 0.4-0.55) in wild SBT and 6.6 ng/g (range 0.81-41) in farmed SBT.

There was a significant relationship between lipid content (% basis) and $\log_{10}$ PCDD/PCDFs and dioxin-like PCBs ($p<0.01$). There was no significant difference in $\log_{10}$ PCDD/PCDFs and dioxin-like PCBs

between wild and farmed SBT (p=0.251). It should be noted that all wild SBT had a low lipid content and low PCDD/PCDFs and dioxin-like PCBs, whilst most of the farmed fish had higher lipid content and PCDD/PCDFs and dioxin-like PCBs. This meant that any differences in PCDD/PCDFs and dioxin-like PCBs between wild and farmed fish might have been confounded with the effect of lipid content. One value for wild tuna was identified as a possible outlier, and when this point was removed and the analysis repeated it was found that there was a significant difference (at the 5% significance level) in $\log_{10}$ PCDD/PCDFs and dioxin-like PCBs between the interaction of lipid content and type (p=0.034). This means that the slope of the fitted line of $\log_{10}$ PCDD/PCDFs and dioxin-like PCBs on lipid content is different for farmed and wild SBT.

The mean concentration of total mercury in wild Australian SBT found in this study was 0.36 mg/kg (range 0.31-0.41) while in farmed SBT it was 0.31 (range 0.18-0.45) (Table 4). All SBT reported in this study had undetectable residues of the metals including antimony, cadmium and lead less than the LOQ of 0.01 mg/kg and for tin less than 0.02 mg/kg. The mean concentration of copper, mercury, and selenium concentration appeared to be lower in farmed SBT than wild caught SBT (Table 4). While for arsenic, there is an increase in farmed SBT concentrations compared to wild SBT. Again, this metalloid may reflect the higher lipid content of the farmed SBT (Table 3).

There was no quantifiable residue of any antimicrobial or any pesticide present in any SBT sample (Tables 1 and 2).

Table 1. Summary of organochlorine and organophosphate pesticide analyses undertaken including Australian and Japanese regulatory limits. All units mg/kg (f.w. basis).

Pesticide	LOQ	Australian MRL/ERL	Japanese MRL[1]
Aldrin	0.01	0.1	0.1
alpha BHC	0.01	0.01	Not set
beta BHC	0.01	0.01	Not set
delta BHC	0.01	0.01	Not set
gamma BHC (Lindane)	0.01	1	0.1
Total BHC	0.01	1	Not set
cis Chlordane	0.01	0.05	0.05
oxy Chlordane	0.01	0.05	0.05
trans Chlordane	0.01	0.05	0.05
Total Chlordane	0.01	0.05	0.05
DDD	0.01	1	3
DDE	0.01	1	3
DDT	0.01	1	3
Total DDT	0.01	1	3
Dicofol	0.01	d biphenNot set	Not set
Dieldrin	0.01	0.1	0.1
Endrin	0.01	Not set	0.05
alpha Endosulfan	0.01	Not set	0.004
beta Endosulfan	0.01	Not set	0.004
Endosulfan Sulphate	0.01	Not set	0.004
Total Endosulfan	0.01	Not set	0.004
Heptachlor	0.01	0.05	0.05
Heptachlor epoxide	0.01	0.05	0.05
Total Heptachlor	0.01	0.05	0.05
Hexachlorobenzene (HCB)	0.01	0.01	0.1
Methoxychlor	0.01	Not set	Not set

Pesticide	LOQ	Australian MRL/ERL	Japanese MRL[1]
Azinphos ethyl	0.01	Not set	Not set
Azinphos methyl	0.05	Not set	Not set
Bromophos ethyl	0.01	Not set	Not set
Carbophenthion	0.01	Not set	Not set
Chlorpyriphos	0.01	Not set	Not set
Chlorpyriphos methyl	0.01	Not set	Not set
cis Chlorfenvinphos	0.01	Not set	Not set
Coumaphos	0.01	Not set	Not set
Demeton-S-methyl	0.01	Not set	Not set
Diazinon	0.05	Not set	Not set
Dichlorvos	0.01	Not set	Not set
Dimethoate	0.05	Not set	Not set
Dioxathion	0.05	Not set	Not set
Ethion	0.01	Not set	Not set
Fenamiphos	0.01	Not set	Not set
Fenchlorphos	0.01	Not set	Not set
Fenitrothion	0.01	Not set	Not set
Fenthion	0.01	Not set	Not set
Malathion (Maldison)	0.01	Not set	0.5
Methacrifos	0.05	Not set	Not set
Methamidophos	0.01	Not set	Not set
Methidathion	0.01	Not set	Not set
Mevinphos	0.01	Not set	Not set
Monocrotophos	0.05	Not set	Not set
Omethoate	0.05	Not set	Not set
Parathion	0.01	Not set	Not set
Parathion methyl	0.01	Not set	Not set
Phosalone	0.05	Not set	Not set
Phosmet	0.05	Not set	Not set
Pirimiphos methyl	0.01	Not set	Not set
Profenofos	0.01	Not set	Not set

Pesticide	**LOQ**	**Australian MRL/ERL**	**Japanese MRL[1]**
Temephos	0.01	Not set	Not set
Total Chlorfenvinphos	0.01	Not set	Not set
trans Chlorfenvinphos	0.01	Not set	Not set
Triazophos	0.05	Not set	Not set
Trichlorfon	0.05	Not set	0.04
Vamidothion	0.01	Not set	Not set

LOQ: Limit of Quantification.

MRL: Maximum Residue Limit as set by Food Standards Australia New Zealand.

ERL: Extraneous Residue Limit as set by Food Standards Australia New Zealand.

[1]MRL: Maximum Residue Limit as set by the Japanese Ministry of Health, Labour and Welfare.

Table 2. Summary of antimicrobial analyses undertaken on farmed Southern Bluefin Tuna.

Antimicrobial compound	LOQ	Australian MRL/ERL	Japanese MRL[1]
Beta-lactams			
Penicillin G	0.01	Not set	Not set
Ampicillin	0.01	Not set	0.06
Amoxicillin	0.01	Not set	0.2
Cloxacillin	0.1	Not set	0.3
Aminoglycosides			
Neomycin	0.1	Not set	0.5
Strepomycin	0.1	Not set	Not set
Dihydrostreptomycin	0.1	Not set	Not set
Apramycin	0.5	Not set	Not set
Gentamicin	0.1	Not set	Not set
Sulphonamides			
Sulphadiazine	0.05	Not set	Not set
Sulphadimidine	0.05	Not set	Not set
Sulphadoxine	0.05	Not set	Not set
Sulphaquinoxaline	0.05	Not set	Not set
Sulphatroxazole	0.05	Not set	0.1
Sulphafurazole	0.05	Not set	Not set
Tetracyclines			
Chlortetracycline	0.05	Not set	0.2
Tetracycline	0.05	Not set	0.2
Oxytetracycline	0.05	Not set	0.2
Macrolides			
Erythromycin	0.1	Not set	0.06
Tilmicosin	0.2	Not set	0.05
Tylosin	0.1	Not set	0.1
Others			
Lincomycin	0.1	Not set	0.05

LOQ: Limit of Quantification.

continued

MRL: Maximum Residue Limit as set for fish by Food Standards Australia New Zealand.

ERL: Extraneous Residue Limit as set for fish by Food Standards Australia New Zealand.

[1]MRL: Maximum Residue Limit as set for fish by the Japanese Ministry of Health, Labour and Welfare.

Table 3. Mean and range of dioxins (PCDD/PCDFs) and polychlorinated biphenyls (PCBs) concentrations in Australian wild caught and farmed Southern Bluefin Tuna on a concentration and Toxic Equivalent (TEQ) basis.

Sample	***n***	**Lipid (%)**	**PCDD/PCDFs (WHO TEQ pg/g)**		**PCBs (WHO TEQ pg/g)**		**PCBs[1] (ng/g)**		**Total PCDD/PCDF and dioxin-like PCBs (WHO TEQ pg/g)**	
Reporting basis[1]	N/A	N/A	f.w.	l.w.	f.w.	l.w.	f.w.	l.w.	f.w.	l.w.
Australian ML	N/A	N/A	Not set	Not set	Not set	Not set	500	Not set	Not set	Not set
Japanese MAL	N/A	N/A	Not set	Not set	Not set	Not set	500	Not set	Not set	Not set
Wild Southern Bluefin Tuna	5	0.90 (0.28-2.30)	0.14 (0.07-0.32)	35 (3.2-120)	0.13 (0.1-0.21)	24 (9.2-45)	0.47 (0.4-0.55)	100 (24-200)	0.27 (0.18-0.45)	60 (12-162)
Farmed Southern Bluefin Tuna	26	11 (0.86-18)	0.20 (0.06-0.80)	2.4 (0.83-7.0)	0.80 (0.17-3.5)	8.3 (2.1-20)	6.6 (0.81-41)	64 (7.7-240)	1.0 (0.23-4.3)	11 (3.0-27)

[1]PCBs: Summation of 45 congeners including the non-ortho and mono-ortho PCBs.

Figures in parentheses indicate the range.

n: number of samples.

N/A: not applicable.

continued

ML: Maximum Level as set for fish by Food Standards Australia New Zealand.

f.w.: fresh weight.

l.w.: lipid weight.

MAL: Maximum Allowable Limit as set by the Japanese Ministry of Health, Labour and Welfare.

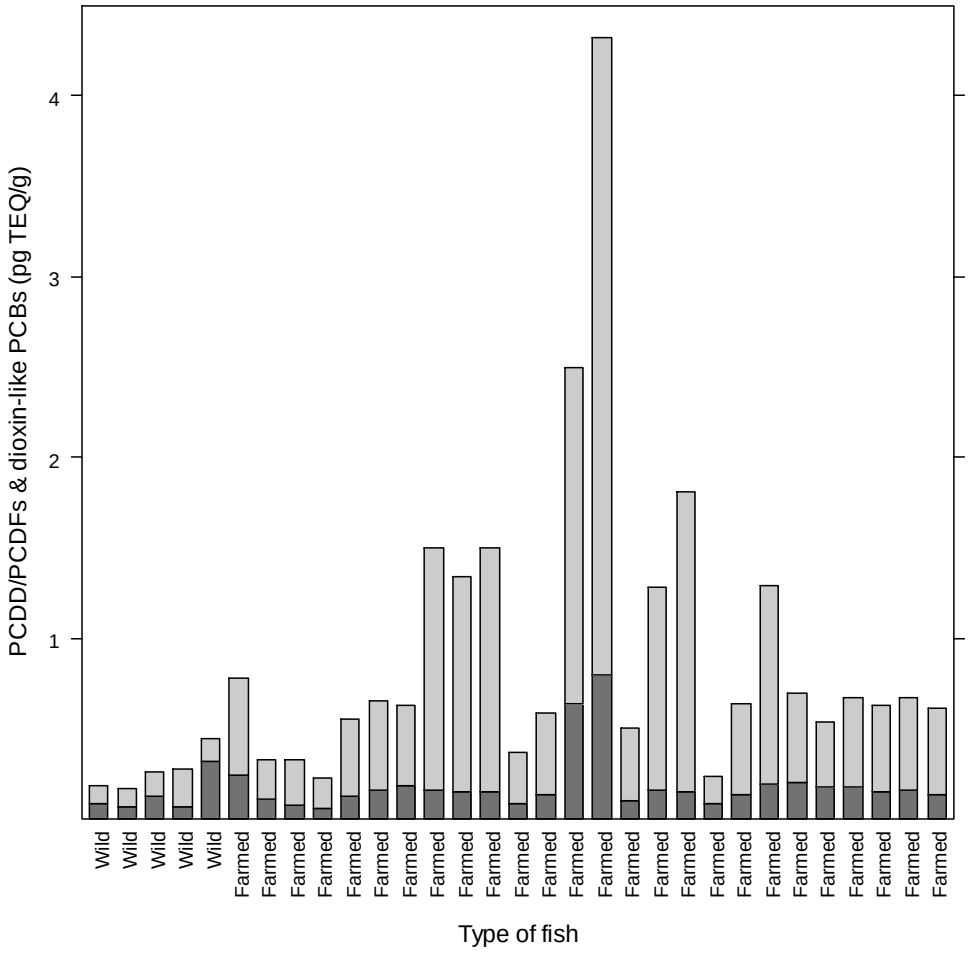

Figure 3. Summed PCDD/PCDFs and dioxin-like PCBs concentrations (pg TEQ/g) in wild caught and farmed Australian Southern Bluefin Tuna. The lower portion of each bar represents the PCDD/PCDF concentration while the upper portion represents the dioxin-like PCBs. All results expressed on a fresh weight and upper bound basis.

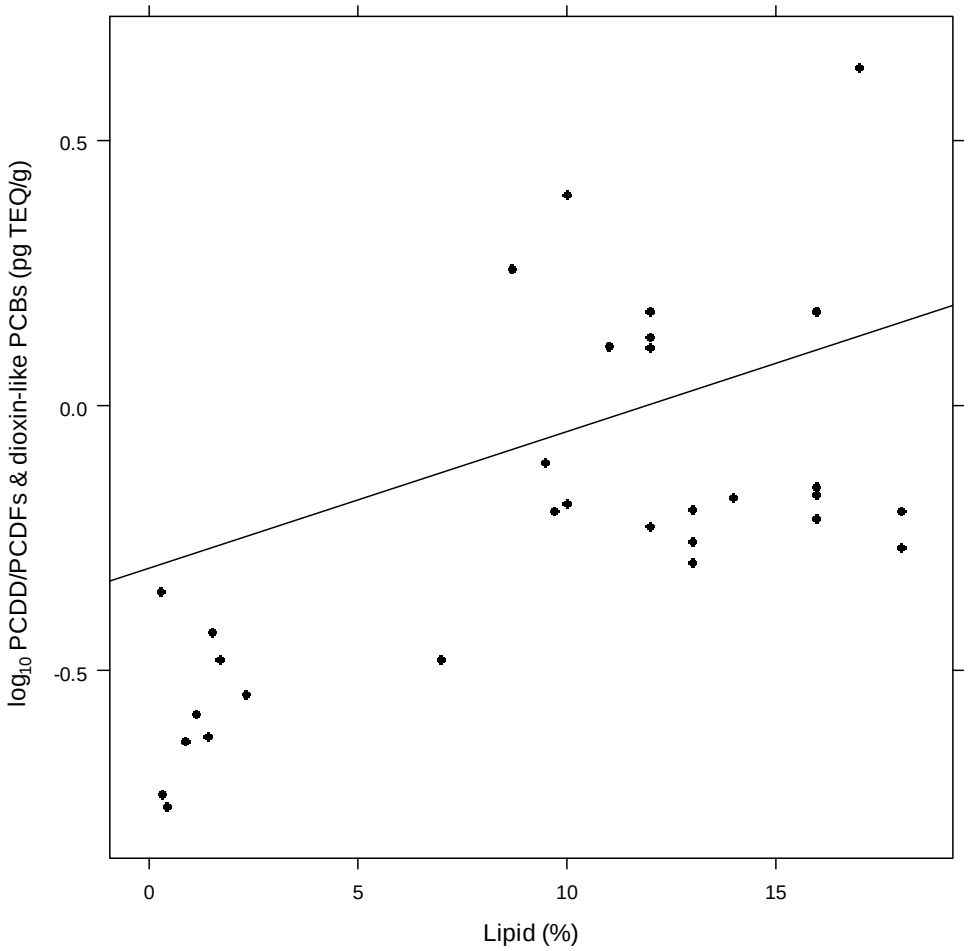

Figure 4. Relationship between total (summed) PCDD/PCDFs and dioxin-like PCBs vs. total lipid content in wild caught and farmed Australian Southern Bluefin Tuna (showing line of best fit). There was a significant relationship between lipid content (% basis) and total (summed) log_{10} PCDD/PCDFs and dioxin-like PCBs ($p<0.01$). All results expressed on a fresh weight upper bound basis.

Table 4. Mean and range of concentrations of metals and metalloids in Australian wild caught and farmed Southern Bluefin Tuna. All units mg/kg (f.w. basis).

Sample	*n*	Metals							Metalloids	
		Antimony (Sb)[4]	Cadmium (Cd)	Copper (Cu)	Lead (Pb)[4]	Mercury (Hg)	Tin (Sn)[4]	Zinc (Zn)	Arsenic (As)	Selenium (Se)
LOQ	N/A	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.02	0.05
Australian ML	N/A	Not set	Not set	Not set	0.5	1	250[1]	Not set	Not set[3]	Not set
Japanese MAL	N/A	Not set	Not set	Not set	Not set	Exempt	Not set	Not set	Not set	Not set
Wild Southern Bluefin Tuna	5	Not measured	<0.01	0.36 (0.31-0.41)	<0.01	0.34 (0.28-0.42)	Not measured	5.0 (4.9-5.2)	0.57 (0.46-0.72)	1.3 (1.1-1.6)
Farmed Southern Bluefin Tuna	26	<0.01	<0.01	0.30 (0.19-0.51)	<0.01	0.31 (0.18-0.45)	<0.02	5.0 (4.0-8.0)	0.71 (0.16-1.8)	0.93 (0.62-1.1)

[1]The ML for Sn only applies to canned food.

[2]Results were all less than the Limit of Quantification (LOQ).

Continued

[3]The ML for arsenic in fish applies to inorganic arsenic (2 mg/kg). This study measured total arsenic.

[4]There was no quantifiable concentration of Sb, Pb or Sn present in any sample.

Figures in parentheses indicate the range.

n: number of samples.

N/A: not applicable.

LOQ: Limit of Quantification.

ML: Maximum Level as set for fish by Food Standards Australia New Zealand (FSANZ).

MAL: Maximum Allowable Limit as set by the Japanese Ministry of Health, Labour and Welfare.

4. Discussion

The residue content of all SBT from this study was evaluated against domestic Australian and Japanese regulatory standards. All SBT from this study were clearly able to satisfy Australian and Japanese regulatory requirements for all residues and contaminants reported regardless of whether they were from wild caught or farmed sources. These standards may be numerically equivalent, but are often technically different in nature. For example, all wild and farmed SBT from this study had PCB residues less than 1/10th of the Australian ML (0.5 mg/kg) (f.w. basis) as set by the Australian Competent Authority Food Standards Australia New Zealand (FSANZ) (Table 3). The Japanese Maximum Allowable Limit (MAL) for PCBs set by the Japanese Ministry of Health, Labour and Welfare (MHLW) is also set at 0.5 mg/kg (f.w. basis). However, the technical basis of these standards differs in that the Australian ML is based on technical mixtures of Aroclor PCBs (1254 and 1260); while in Japan the MAL is based on technical mixtures of Kaneclor PCBs (300, 400, 500 and 600).

From Table 3, the mean concentration (f.w. basis) of PCDD/PCDFs and dioxin-like PCBs found in Australian farmed SBT was 0.97 pg TEQ/g (range 0.23-4.3); in wild SBT it was 0.27 pg TEQ/g (range 0.18-0.45). The lipid content in wild SBT was less than 1/10th of that found in farmed SBT. Concentrations of PCDD/PCDFs and PCBs reported differed between individual SBT with greater variability observed in farmed than in wild SBT. This difference is attributed to differences in lipid content between wild and farmed SBT in addition to the larger sample numbers for farmed SBT (Table 3). No information is available on the

genetic history or relatedness of the SBT in this study; apparent clustering observed could be of a genetic origin or simply related to age of fish sampled or other factors such as feed history, culture period and gender (Figure 4). Other explanations as to why these contaminants did not appear to be retained in the SBT could be due to the homogeneity of the feed samples taken or a rapid turnover of individual feed batches or that SBT were possibly consuming other wild fish living in the sea-cages. The PCDD/PCDF and dioxin-like PCB profile present was dominated by the dioxin-like PCBs (Figure 3). It is speculated that the principal source of these PCDD/PCDFs and dioxin-like PCBs is from baitfish feeds. Supporting this assertion, seafloor sediment samples were collected from Fitzgerald Bay and Port Pirie within the Spencer Gulf as part of the National Dioxins Program in 2004. Concentrations of PCDD/PCDFs and dioxin-like PCBs (medium bound basis) found were 0.18 pg TEQ/g and 0.17 pg TEQ/g (dry weight basis) for Fitzgerald Bay and Port Pirie respectively (Müller et al., 2004). Based on these results, it is unlikely that the Spencer Gulf seafloor environment is a major source of these contaminants in farmed SBT. Seawater is a potential source of PCDD/PCDFs in wild capture baitfish species such as Australian sardine (*Sardinops sagax* - Steindachner) caught in the Spencer Gulf for use as feed for captive SBT; previous investigation has found negligible background levels in mussels and seafloor sediment from the Spencer Gulf region of SA (Müller et al., 2004). Baitfish species imported from elsewhere in the world may be harvested from seawater containing greater levels of these contaminants in response to local sources.

Concentrations of PCDD/PCDFs and PCBs have been reported in Pacific Bluefin Tuna (*Thunnus thynnus*) purchased from Spanish retailers over the years 1995 to 2003. Summed PCDD/PCDF and PCB concentrations (f.w. basis) for these fish (n=4) ranged between 0.41-7.5 pg TEQ/g (Gómara et al., 2005). Mid-dorsal samples of Bluefin Tuna (*Thunnus thynnus*) from the Italian Egadi Islands near Sicily were analysed for the dioxin-like PCBs (Corsolini et al., 1995). The mean weight of these tunas was 320 kg (range 70-400). The mean total PCB concentration (f.w. basis) reported was 0.85 mg/kg (range 0.17-2.2) and the dioxin-like PCBs was 77 pg TEQ/g (range 17-200) (lower-bound basis). The concentration of these compounds in Australian wild and farmed SBT were considerably lower than those reported from the Egadi Islands and Spain (Table 3). The Kaneclor PCB content of liver samples of Japanese wild caught Bluefin Tuna (*Thunnus thynnus*) up to 3.9 mg/kg on a lipid weight (l.w.) basis has been reported in a male fish with a body length of 1.86 m, body weight of 135 kg and lipid content of 12 % (Ueno et al., 2002). Farmed fish have been observed to be able to excrete PCDD/PCDFs and PCBs in addition to growth dilution of these compounds (Brambilla et al., 2007). The main source of these residues in farmed fish is believed to be from feeds (Jacobs et al., 2002).

PCDD/PCDF and PCB data from harvested bluefin tunas from capture-based aquaculture systems elsewhere in the world are often of limited value. Previous data have been published for Australian farmed SBT (Padula et al., 2004). The comparability of data from other studies is often constrained due to a number of technical inconsistencies including:

1. Selective reporting of tissue(s) analysed,
2. Inclusion of bones and or skin in samples tested,
3. Differing TEF values used for calculation of TEQs,
4. Treatment of non-detects and laboratory blanks,
5. Reporting of upper, medium and lower bound data,
6. Reporting results for non-edible tissues,
7. Reporting of selected PCB or PCDD/PCDF congeners,
8. Differing Limits of Detection (LODs) and LOQs
9. Differing analytical methodologies,
10. Fresh and lipid weight reporting of results,
11. Failure to report the lipid content of samples, and
12. Failure to report the weight or fork length of fish sampled.

Published data are of limited value to other researchers if data are not reported in an internationally consistent manner.

The European Union (EU) new MLs for the sum of PCDD/PCDFs and the dioxin-like PCBs of 8 pg TEQ/g (based on 1998 TEF values) came into effect on November 4th 2006. All SBT from this study were clearly able to comply with this and the existing PCDD/PCDF ML of 4 pg TEQ/g. Lipid-based results for PCDD/PCDFs and PCBs were higher in wild SBT compared to farmed SBT (Table 3). The dominant contribution to the concentrations (TEQ basis) reported was from the dioxin-like PCBs. The dominance of the dioxin-like PCBs in SBT may represent physiological processes related to lipid deposition and feeding of different baitfish feeds. The publication of revised TEF

values for mammalian reporting will create difficulties for future researchers as previously published data will not be comparable with new data due to differences in the TEF values.

Mercury is present in several chemical forms in fish; the most toxic being alkylated organic mercury forms such as methylmercury. The ratio of methylmercury (MeHg) to total mercury is related to trophic position in the marine food chain (Dietz et al., 2000). The mean concentration of total mercury in wild Australian SBT from this study was 0.36 mg/kg (range 0.31-0.41) while in farmed SBT it was 0.31 (range 0.18-0.45) (Table 4). Total mercury content of wild Bluefin Tuna (*Thunnus thynnus*) (n=7) from the Ionian Sea has been reported in fish (weight range 2.85-4.36 kg and fork-length range 54-62.5 cm) as ranging between 0.13-0.35 mg/kg (Storelli et al., 2005). All SBT reported in this study had concentrations of antimony, cadmium and lead less than the LOQ of 0.01 mg/kg and for tin less than 0.02 mg/kg. There is an Australian ML set for lead in fish of 0.5 mg/kg. There are no Australian MLs set for antimony or cadmium in fish.

In Japan all *Thunnus spp*. are exempt from the MALs set for mercury in the *Provisional Regulatory Limitations for Contaminants in Foods* within the *Food Sanitation Law* administered by MHLW. Recently the Japanese Food Safety Commission released its study: *Food Safety Risk Assessment Related to Methylmercury in Seafood* in response to a request from MHLW to undertake a risk assessment of methylmercury in seafood (Japanese Food Safety Commission, 2005). The lack of a standard analytical method for the analyses of methylmercury in seafood means there is

great variation in ratios of methylmercury to total mercury reported. Canned bluefin tuna (species unknown) has been reported as having a MeHg to total mercury ratio of 78 % (Cappon and Smith, 1981). This study did not measure MeHg due to lack of accredited analytical laboratory capability in Australia. The Australian Competent Authority, Food Standards Australia New Zealand (FSANZ) assumes all mercury present in seafood is present as MeHg when undertaking dietary modelling and risk assessments.

Antimicrobial residues were not found in any farmed SBT tested (Table 2). Farmed SBT are not affected by bacterial diseases of production importance (Munday et al., 2003). This may reflect the low stocking densities, immunocompetence of the captive fish and presence of few human settlements near the culture area. It is believed the main reason why SBT are not affected by diseases during culture is because only adult fish which have already been exposed and survived infections in the wild and so they are immune to future challenges during farming. Antibiotics in the marine environment may also arise from runoff from terrestrial agriculture or sewage treatment plants (Hirsch *et al*., 1999). In Australia, prescribing authority for administration of veterinary medicines to food producing animals is restricted to authorised registered veterinary practitioners. Veterinary medicine registration for use in food producing animals is nationally controlled by the Australian Pesticides and Veterinary Medicines Authority (APVMA). There are no Australian regulatory standards set for the presence of any of the antimicrobial residues analysed for in this study.

The absence of pesticides from any SBT tested may reflect baitfish feed sourcing strategies employed by the SBT industry and the introduction of pre-testing vendor declaration arrangements between baitfish suppliers and SBT companies. Many persistent organochlorine pesticides such as DDT have been banned in Australia since 1987 (Connell et al., 2002). Many of the pesticide compounds analysed for do not have Australian regulatory standards set for their presence in fish. Australia has two forms of nomenclature for regulatory limits for agricultural chemical residues in foods: Maximum Residue Limits (MRLs) and Extraneous Residue Limits (ERLs). An MRL is set for agricultural chemicals that have registration for usage in Australian agriculture, while an ERL is set for agricultural chemicals such as DDT whose registration has been cancelled, but their presence may persist in the environment due to historical usage. The metabolism of pesticides by marine bacteria may produce intermediate metabolite compounds that may be more stable than the parent compound such as *p,p'*-DDE the metabolite of DDT (Connell et al., 2002). The arid Spencer Gulf catchment area is largely under dryland farming production for grains and cereal crops.

The product integrity and safety of Australian wild-capture farmed SBT has been demonstrated through this study for a range of natural and environmental contaminants. There is a need to investigate the effect of feed history and culture period on concentrations of these residues and contaminants in farmed SBT. This will help to develop risk management approaches for aquaculture production systems. There is growing awareness of residues in seafood in international trade and

there is a need for harmonisation of frontier border inspection systems. The use of the standardised sampling method in this study adapted from the Japanese MAFF frontier border inspection system is one example of international trade harmonisation efforts. Further work will need to be undertaken on the harmonisation of analytical methods through international fora such as the Codex Alimentarius Commission (CAC) and World Trade Organisation (WTO).

5. Conclusion

The product integrity status of Australian SBT was acceptable for market access needs with all residues and contaminants measured, either absent, or present at acceptable levels as judged by Australian and international food regulatory standards. The dioxin-like PCBs deserve further detailed attention in future studies to understand their relative large contribution to the total TEQ. This will require investigation into the presence of these contaminants in feeds, impact of culture period duration and the distribution of these contaminants within farmed SBT. Future work will help to build risk management tools to control these residues and contaminants in farmed SBT to meet emerging trade access requirements and the expectations of food safety aware consumers. Analytical capability for mercury speciation would allow risk characterisation of mercury compounds present in SBT to better inform risk management decision makers. SBT also contains a range of minerals of public health importance. The effect of inconsistent adoption of international residue and contaminant nomenclature coupled with differing reporting conventions creates ambiguity for risk management decision making. This coupled with differing sample

collection and processing methods may lead to different consumer interpretations of the safety of SBT in different markets. The work undertaken here provides a scientific basis for product safety differentiation against other *Thunnus* spp. in markets entered by SBT.

Acknowledgements

Part of this study was undertaken for the National Dioxins Program through the Australian Government Department of the Environment and Heritage (DEH) in conjunction with the National Residue Survey within the Australian Government Department of Agriculture, Fisheries and Forestry (DAFF). This work formed part of a project of the Aquafin Cooperative Research Centre (CRC) and received funds from the Australian Government's CRCs Program, the Fisheries Research and Development Corporation (FRDC) and other CRC Participants. Mr. Andrew Pointon (South Australian Research and Development Institute) and Mr. Steven Clarke (South Australian Research and Development Institute) are both thanked for reviewing this manuscript. Ms. Rose Flint (South Australian Resarch and Development Institute) is thanked for assistance with statistical analysis and preparation of figures. Mr. Thomas Madigan (South Australian Research and Development Institute) is thanked for technical project support.

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CHAPTER FOUR

Accepted for publication manuscript

Padula, D.J.; Madigan, T.L.; Nowak, B.F. Australian farmed Yellowtail Kingfish (*Seriola lalandi*) and Mulloway (*Argyrosomus hololepidotus*): Residues of metallic, agricultural and veterinary chemicals, dioxins and polychlorinated biphenyls. Accepted for publication in Chemosphere.

1. Introduction

The positive public health benefits of regular seafood consumption are acknowledged, however, international public attention has been drawn to the presence of a wide range of pollutants of public health concern in farmed seafood (Tanabe 1988; Zhang et al., 1999; Jacobs et al., 2002; Hites et al., 2004; European Food Safety Authority, 2005; Domingo, 2007a; Domingo, 2007b; Domingo, 2007c; Martı´-Cid et al., 2007; Kelly et al., 2008; Sapkota et al., 2008; Kleter et al., 2009). Pollutants may be present in seafood for a range of reasons, including natural occurrence (mercury from seabed volcanic eruptions), veterinary treatments or inadvertent human sources such as high temperature waste incineration(Kleter et al., 2009; McKay, 2002). Advances in analytical instrumentation have led to the discovery of an increasing range of these compounds, previously not known to exist in food (Díaz-Cruz and Barceló a, 2007; Herzog, 1970). Recent food-related contamination incidents have affected public confidence, prompting greater scrutiny of these residues and contaminants in common foodstuffs (Huang et al., 2006). This has translated into greater scrutiny of aquaculture products at frontier border inspection stations in some overseas markets (Knight et al., 2008). When regulatory standards are exceeded in domestic or international markets, or when a new public health threat is identified for a compound with no prior food regulatory history, major disruptions in the commerce of these foods can occur (Ababouch, L. 2006; Anon., 2009).

Maximum Residue Limits (MRLs) are regulatory standards for foods that set the maximum level of a chemical residue that is permitted to be present in a food (Hyder et al., 2003). An MRL takes into account biochemistry, metabolism, available analytical methodology, residues, good agricultural practice, toxicology and dietary exposure issues (Abbott et al., 2003). Environmentally persistent chemicals (such as the pesticide DDT) whose Australian registration has ceased are given an Extraneous Maximum Residue Limit (EMRL) in Australian food standards (Abbott et al., 2003). Harmonisation of food regulatory standards between trading partner nations, using internationally agreed risk analysis protocols (e.g. internationally traded commodities such as the two species aquaculture fish species in the present study), is the main focus of bodies such as the Codex Alimentarius Commission (Hathaway, 1993).

Yellowtail Kingfish (*Seriola lalandi* - Valenciennes, 1833) (YTKF) is a marine finfish species of economic importance for the South Australian (SA) aquaculture industry. YTKF aquaculture began in SA in the mid 1990s, while aquaculture production of Mulloway (*Argyrosomus hololepidotus* - Lacépède, 1802) began more recently, in approximately 2000 (Primary Industries and Resources South Australia, 2011; Poortenaar, 2001). Both species are produced commercially in SA through two hatcheries located in the Spencer Gulf region at Arno Bay and Port Augusta, with aquaculture grow-out occuring at Arno Bay (one producer) and Whyalla (two producers) (Figure 1). In 2007/2008 the net total economic impact of these two marine finfish species to SA

was $58,000,000 (Primary Industries and Resources South Australia, 2011).

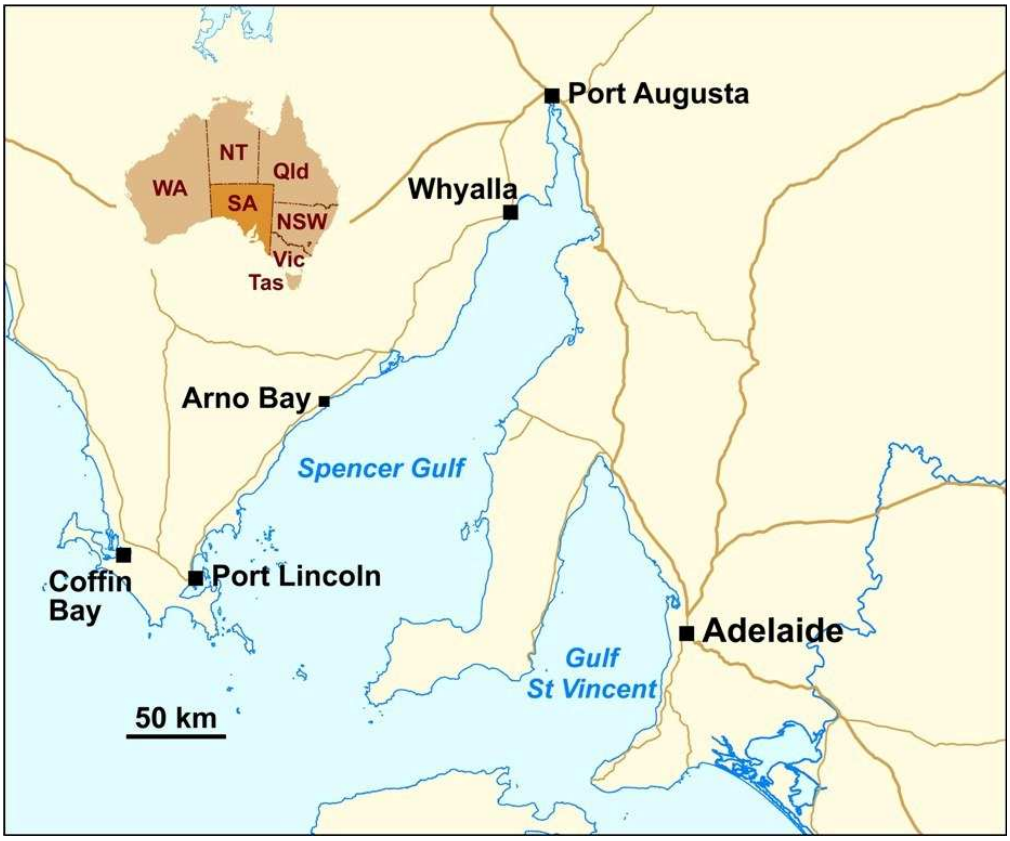

Figure 1. Map of the Spencer Gulf region of South Australia (SA). The townships of Arno Bay, Whyalla and Port Augusta are SA production sites of Yellowtail Kingfish. Mulloway is only produced at Arno Bay.

Previous contamination investigation studies of the Spencer Gulf marine environment have focused on specific contaminants in sediments, including dioxins and PCBs (Müeller et al., 2004). These studies have reported the presence of only background levels of these organochlorine contaminants (Müeller et al., 2004). However, other sources of contaminants can also affect aquaculture products. For

example, feed and antifouling paints may be sources of pollutants in farmed fish (Fernandes et al., 2009).

Fat soluble contaminants, such as dioxins, PCBs and other organochlorine compounds, are generally of human origin and are mostly concentrated around populated areas as a consequence of activities such as high temperature municipal and medical waste incineration, paper manufacture, copper and iron ore smelting. (MacLachlan and Bhula, 2009). Due to their environmental stability, these compounds may persist for many years following application (such as for pesticides), even after product registration has ceased (Tanabe, 1998; Connell et al., 2002).

Metallic contaminants, such as mercury, have biomagnification potential in seafood but are concentrated in protein containing tissue (Balshaw et al., 2007). Elements such as mercury have also been reported as affecting fish health (Protasowicki, 1991). Generally, metallic contaminants are of natural origin and arise from activities such as weathering of geological features, but some may have had historical use as active ingredients in crop protection chemical or arise from fossil fuel combustion (Balshaw et al., 2007; Pyle and Mather, 2003). Some metalloids such as inorganic arsenic compounds (arsenicals) had historical usage as ingredients in veterinary anthelmintic preparations (McKellar and Jackson, 2004). Veterinary medicines vary in their accumulation potential in aquatic animals and currently there is little published information on usage of these products in YTKF, Mulloway or related species worldwide (Katae et al.,

1980; Ohno et al., 2009). Japanese farmed Yellowtail (*Seriola quinqueradiata*) is known to be affected by a number of diseases of economic importance; Western Australian farmed YTKF have been affected by a mass mortality incident (Egusa, S. 1983; Stephens and Savage, 2010).

So far, no studies have been published on the residue status of either of these two new Australian aquaculture species that demonstrate their safety to Australian and international consumers on a through chain basis as has been undertaken with Australian terrestrial food producing animals (Pointon et al., 2006). Only a single study has previously reported metallic contaminants in Australian wild capture YTKF (Chvojka, 1988). This study provides additional and up-to-date data that may be useful in market access negotiations.

2. Aim of study

The aim of this study was to benchmark in Australian farmed YTKF and Mulloway, levels of a broad range of residues and contaminants of potential public health and trade significance in relation to international market access.

3. Materials and methods

3.1 Sample collection

YTKF (n=135 individual fish were used to form n=27 pooled samples for laboratory testing) were collected from all three SA producers, while Mulloway (n=30 individual fish were used to form n=6 pooled samples for laboratory testing) were obtained from the single SA

producer (this producer also produced YTKF which were tested). The farm sites for fish collected in this study were Arno Bay and Whyalla in the Spencer Gulf region of SA (Figure 1). Fish were matched for size and only pooled from the same sea-cages. Fish were obtained as whole gilled and gutted (head on) from each of the companies in this study during commercial harvests. Each of the three aquaculture establishments produce fish for different markets and customer requirements e.g. cooked fish, raw sushi and sashimi fish and for value-added products. Fish taken as part of the present study were representative in size of fish commercially traded domestically and internationally. All samples, including Australian manufactured aquaculture feed (n=5), were collected between September 2003 and July 2004. Feed samples (1 kg) were drawn from batches of aquaculture feeds being fed at each of the three establishments on the day of sample collection (each of the establishments were supplied with feed from both Australian aquaculture feed millers). The feeds (steam extruded pellets) tested were manufactured in Australia by two aquaculture feed millers (designated by A or B in Figure 1). Both Australian feed millers operate under Hazard Analysis and Critical Control Points (HACCP) programs as defined by CAC/RCP 1-1969 (Rev.4-2003) and AS/NZS ISO 9001:2000 for manufacture of extruded fish food.

3.2 Sample processing

Fork length and weight of each fish was recorded. Pooled samples were formed from fillets taken from both sides of five individual fish from a single establishment. The skin, bones and dark meat were removed from each fillet and discarded. These pooled fillets were homogenised

in a stainless steel HOBART™ food processor. Manufactured feed pellets (one kg) were homogenised using the same food processor.

All equipment, tables, benches, knives, food processor and implements were cleaned with DECON90 laboratory detergent and double rinsed with mains tap water before and after each sample was processed. Samples for dioxin and PCB analyses were wrapped separately in aluminium foil sheeting provided by the contract laboratory. All sample bags were double bagged to protect sample integrity and stored at –40˚C until despatch to the laboratories for analysis (no longer than 30 days). Samples were couriered to the laboratories in a sealed foam box in which several freezer gel packs were placed. Laboratories stored samples at -20˚C upon arrival. Samples were thawed at 4˚C for 24 hours, with all thawing juices included in the defrosted analytical sample. Analysis began one day post arrival of samples at each laboratory.

3.3. Chemical analyses - PCDD/PCDFs and PCBs

PCDD/PCDF and PCB analyses were undertaken by a New Zealand laboratory accredited by International Accreditation New Zealand (IANZ) to ISO/IEC 17025 (1999) (chemical accreditation held in "foodstuffs" matrices).

3.3.1 Sample Preparation

Samples (40 g) were blended with powdered sodium sulfate (BDH, analytical reagent grade) and loaded into a Soxhlet extractor (made by an in-house glass blower), fortified with labeled internal standards

(EPA-1613LCS and 68A-LCs, Wellington Laboratories) and extracted with organic solvent (methylene chloride:hexane 1:1) (Mallinckrodt Ultim, analytical reagent grade) for 16 hours. A clean-up and recovery standard was then added, the extract was evaporated to constant weight and the lipid weight determined gravimetrically. Clean-up was undertaken by solid phase (in-house prepared columns) clean-up techniques using acid and base modified silica gel (silica gel 60, Merck). The extract was purified with column chromatography techniques using activated alumina (aluminium oxide 90 active acidic, Merck) and carbon (carbopack C 60/80 mesh, Supelco). The final extract was concentrated and fortified with recovery standards (EPA-1613ISS and 68A-ISS, Wellington Laboratories) and analysed by High Resolution Gas Chromatography - High Resolution Mass Spectroscopy (HRGC-HRMS).

3.3.2 PCDD/PCDFs and PCB determination

Analysis followed United States Environmental Protection Agency (USEPA) method 1613B for PCDD/PCDFs and USEPA method 1668A for PCBs. It was carried out on a Micromass Autospec Ultima High Resolution Mass Spectrometer (United Kingdom) interfaced to an Agilent 6890 chromatograph (United Kingdom) operating in the splitless mode, equipped with Zebron ZB-5 capillary columns for PCB and PCDD/PCDF. HRMS analysis was carried out in the electron impact mode. Native and labeled compounds were acquired by Selected Ion Monitoring (SIM) with the mass resolution being maintained at 10,000 (5% valley) throughout the analysis.

Chromatographic data were processed using QuanLynx™ (Waters MassLynx™ software package).

Analysis for PCBs included the non-ortho PCB congeners 77, 81, 126, 169 and mono-ortho PCB congeners 105, 114, 118, 123, 156, 157, 167, 189. In addition, a group of indicator PCB congeners was measured: 1, 3, 4, 15, 19, 28, 37, 44, 49, 52, 54, 70, 74, 99, 101, 104, 110, 138, 153, 155, 170, 180, 183, 187, 188, 194, 196, 199, 202, 205, 206, 208 and 209. Concentrations of individual congeners were determined via isotope dilution. Total PCB concentration was determined by the summation of individual concentrations of all detected PCB congeners with non-detects treated as being equal to the Limit of Reporting (LOR).

3.3.3 Quality assurance and quality control

If no peak was distinguishable above the background noise at the retention time for a targeted analyte, or if a peak was present at the correct retention time for the targeted analyte but failed to meet all analyte identification criteria, the result was reported as a limit of detection value. The isotope dilution technique involves determining the native analyte levels using the ratio of responses for the native and internal (isotopically labeled) standards. Hence the native analytes are self-corrected for recovery. Results were reported on a fresh weight (f.w.) and lipid weight (l.w.) basis.

3.3.4 Calculation of PCDD/PCDF and dioxin-like PCB Toxic Equivalents (TEQs)

For PCDD/PCDFs and dioxin-like PCBs, each congener concentration (upper bound basis) was first standardised to its 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) TEQ concentration (that is TCDD has a value of 1). The TEQ is formally defined as (Van den Berg et al., 1998):

$$TEQ = \sum_{n1}[PCDD_i \times TEF_i] + \sum_{n2}[PCDF_i \times TEF_i] + \sum_{n3}[PCB_i \times TEF_i] \quad (1)$$

where

- TEQ is Toxic Equivalent concentration;
- n1, n2 and n3 are the number of PCDD, PCDF and PCB congeners in the sample;
- $PCDD_i$, $PCDF_i$ and PCB_i are the concentration of PCDD, PCDF and PCB congener i; and
- $(TEF)_i$ is the human Toxic Equivalency Factor (1998 values used; Van den Berg et al., 1998) value of the specific congener.

3.4 Metallic elements determination

Metallic analyses (Cd, Cu, Pb, Hg, Zn, As and Se) were undertaken by an Australian laboratory accredited by the National Association of Testing Authorities (NATA) to the requirements of ISO/IEC 17025 (1999) (chemical accreditation for trace elements held in "seafoods" matrix). An in-house method (NT2.46 Trace metals in food and biota) was used for sample preparation. A subsample (about 1 g) was digested with concentrated nitric acid (Merck, redistilled analytical

reagent grade) and hydrochloric acid (Merck, redistilled analytical reagent grade) for two hours in a 95-100°C water bath. Analysis was based on USEPA methods 6010, 6020 and AOAC Methods 986.15, 974.14 (16th Edition). Analysis was performed by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) on an Elan 6100 DRC (Perkin Elmer, United States).

3.4.1 Quality assurance and quality control

For every 20 samples one blank, one duplicate, one blank spike, one sample matrix spike and one in-house prepared laboratory control sample (prawn tissue) were run by the contract laboratory. Recoveries for al laboratory in-house control and spiked samples (including spiked blanks) were all between 80-120 %. Duplicates all had a Relative Percentage Difference (RPD) <24 %. Blanks were all less than the Limit of Reporting (LOR). All results were corrected for recoveries where necessary. All recoveries met internal NATA method requirements.

3.5 Pesticide determination

Pesticide analyses were undertaken by an Australian laboratory accredited by the National Association of Testing Authorities (NATA) to the requirements of ISO/IEC 17025 (1999) (chemical accreditation for pesticides held in "meat fat and miscellaneous foods matrices). Sample preparation was undertaken in accordance with an in-house method (NR36 Analysis of multi residues by GC/MS organochlorines, organophosphates, PCBs, fungicides, herbicides, synthetic pyrethroids and carbamates). The entire sample was first re-homogenised. A subsample (1 g) of the re-homogenised sample was mixed with

anhydrous sodium sulphate (Ajax, Finechem) and extracted with hexane:acetone 60:40 (Merck, chromatography grade). The extract was cleaned up using Gel Permeation Chromatography (Gilson ASPEC) and the final extract was analysed by Gas Chromatography/Mass Spectroscopy (GC/MS) (Hewlett Packard 5973, United States) in scan mode with a PTV large volume injector (Agilent 5973, United States). The GC/MS pesticide method was adapted from the Pesticides Analytical Manual (FDA, PAM Vol. 1, 3rd edition, 1996). The GC/ECD method followed US EPA 8081. Analytes were quantified using the internal standard method - US EPA 8270 internal standard mix (Supelco, cat 4-8972). Organochlorine analytes were determined by Gas Chromatography (GC) twin column Electron Capture Detector (ECD) (Agilent 6890, United States).

Analyses included the following organochlorine compounds (followed by their respective Limit of Reporting (LOR) in mg/kg) aldrin 0.01, alpha HCH 0.01, beta HCH 0.01, delta HCH 0.01, gamma HCH (Lindane) 0.01, Total HCH 0.01, cis chlordane 0.01, oxy chlordane 0.01, trans chlordane 0.01, total chlordane 0.01, DDD 0.01, DDE 0.01, DDT 0.01, total DDT 0.01, dicofol 0.01, dieldrin 0.01, endrin 0.01, alpha endosulfan 0.01, beta endosulfan 0.01, endosulfan sulphate 0.01, total endosulfan 0.01, heptachlor 0.01, heptachlor epoxide 0.01, total heptachlor 0.01, hexachlorobenzene (HCB) 0.01, methoxychlor 0.01. Analyses included the following organophosphate compounds (followed by their respective LOR) azinphos ethyl 0.01, azinphos methyl 0.05,bromophos ethyl 0.01, carbophenthion 0.01, chlorpyriphos 0.01, chlorpyriphos methyl 0.01, cis chlorfenvinphos 0.01, coumaphos

0.01, demeton-s-methyl 0.01, diazinon 0.05, dichlorvos 0.01, dimethoate 0.05, dioxathion 0.05, ethion 0.01, fenamiphos 0.01, fenchlorphos 0.01, fenitrothion 0.01, fenthion 0.01, malathion (maldison) 0.01, methacrifos 0.05, methamidophos 0.01, methidathion 0.01, mevinphos 0.01, monocrotophos 0.05, omethoate 0.05, parathion 0.01, parathion methyl 0.01, phosalone 0.05, phosmet 0.05, pirimiphos methyl 0.01, profenofos 0.01, temephos 0.01, trans chlorfenvinphos 0.01, total chlorfenvinphos 0.01, triazophos 0.05, trichlorfon 0.05 and vamidothion 0.01.

3.5.1 Quality assurance and quality control

The contract laboratory ran samples in batches of 20 which included a matrix blank, spike and duplicate analyses. The recoveries for spikes were all between 70 and 130%. Blanks were all less than the Limit of Reporting (LOR) for all batches. All results were corrected for recoveries. All recoveries met internal NATA method requirements.

3.5.2. Lipid content determination

Lipid content analysis was undertaken by an Australian laboratory accredited by the National Association of Testing Authorities (NATA) to the requirements of ISO/IEC 17025 (1999) (chemical accreditation for lipid determination held in "biota" matrices). A subsample (5 g) was weighed in a pre-dried soxhlet fat determination thimble. The thimble was placed in a soxhlet apparatus for at least 16 hours using diethyl ether (Merck, analytical reagent grade) on a constant temperature (90°C) water bath. The flask was dried in a convection oven at 102°C for two hours and then cooled in a desiccator to room temperature. The flask was returned to the oven and underwent successive re-weighings

until no further weight loss occurred between successive dryings. The lipid content of the sample was calculated by subtracting the final flask weight from the initial flask weight and dividing this figure by the original sample mass. Results were then expressed as a percentage (%). The method followed AOAC Total Fat Determination by Soxhlet Extraction (16th Ed. 1995).

3.6 Antimicrobial determination – microbial inhibition test (MIT)

Analyses were undertaken for a range of antimicrobial compound classes (beta-lactams, cephalosporins, aminoglycosides, sulphonamides, tetracyclines, macrolides and lincosamides) by an Australian laboratory accredited by the National Association of Testing Authorities (NATA) to the requirements of ISO/IEC 17025 (1999) (chemical accreditation held in "fish and seafoods" matrix). The scope of NATA accreditation held by the laboratory did not include stockfeed, hence it was not possible to test any aquaculture feed samples for the presence of antimicrobial compounds as part of this study. All results were corrected for recoveries.

Analyses included the following antimicrobial compounds (followed by their respective Limit of Reporting (LOR) in mg/kg) beta-lactams included penicillin G 0.01, ampicillin 0.01, amoxicillin 0.01, cloxacillin 0.1. Cephalosporins included ceftiofur 0.2, cefuroxime 0.05, cephalonium 0.05. Aminoglycosides included neomycin 0.1, streptomycin 0.1, dihydrostreptomycin 0.1, apramycin 0.5, gentamicin 0.1, sulphonamides included sulphadiazine 0.05, sulphadimidine 0.05, sulphadoxine 0.05, sulphaquinoxaline 0.05, sulphatroxazole 0.05,

sulphafurazole 0.05, sulphathiazole 0.05, sulphapyradine 0.05, sulphamerazine 0.05, sulphameter (sulphamethoxydiazine) 0.05. Tetracyclines included chlortetracycline 0.05, tetracycline 0.05, oxytetracycline 0.05, doxycycline 0.05. Macrolides and Lincosamides included erythromycin 0.1, tilmicosin 0.2, tylosin 0.1 and lincomycin 0.1.

3.6.1 Betalactams, macrolides/lincosamides, tetracyclines and aminoglycosides

Samples (10 g) were first extracted by high-speed blender with a 1:1 mixture of acetonitrile (Merck, HPLC grade) and methanol (Merck, HPLC grade). The betalactams and macrolide groups are extracted into the solvent mix, whilst the tetracyclines and aminoglycosides bind to the tissue protein precipitate. The protein precipitate was extracted with EDTA (Sigma, analytical grade) solution and a dilute perchloric acid (Sigma, analytical grade) solution. The sample was then put through a C-18 and a SCX cartridge (Varian-Bond Elut) for clean up. This separated the tetracycline and aminoglycoside fractions. All fractions were then plated across multiple microbiological culture plates cultured with antimicrobial sensitive organisms: Cereus plate cultured with *Bacillus cereus*, Thermo plate cultured with *Geobacillus stearothermophilus*, Luteus plate cultured with *Bacillus luteus* and MIT B plate cultured with *Bacillus Subtillus*. MIT B plates were from Silliker Microtech. All other plates were prepared in-house.

Blank controls and spiked plates were run. For MIT screening, three different spikes were performed for each batch of samples. Each of

these three spikes contained representative analytes for each group of antimicrobial compounds. Any detections by MIT were subjected to confirmatory method testing by HPLC and LCMS-MS with all analytes within each antimicrobial compound group spiked at LOR, 2xLOR and 5xLOR levels for each batch of samples.

3.6.2 Sulphonamides

Samples (5g) were extracted using ethyl acetate (Ajax UNIVAR analytical reagent) in a high-speed blender. The ethyl acetate was then reduced by evaporation under nitrogen and then re-extracted in dilute hydrochloric acid (Merck, analytical grade). The sample was then derivatised with fluorescamine before analysis by High Performance Liquid Chromatography (HPLC) (HP Angilent 1100) (reverse phase) with fluorescent detection. External standards (all obtained from Sigma Aldrich except Sulphatroxazole, which was obtained from the Australian Government National Measurement Institute, Pymble, Australia) were used for calibration (0.05, 0.10 and 0.2 mg/kg) and quantitation of all samples and quality control spikes.

3.7 Quality assurance and quality control

Duplicate samples were run every 10^{th} sample or a minimum of one duplicate per batch if <10 samples in the batch. All results were corrected for recoveries, with recovery for each analyte required to fall within +/− 2 SD (Standard Deviation) of Running Mean (RM) recovery. RM recovery is based on an in-house control chart which records all recovery data including during method development and validation. All recoveries met internal NATA method requirements.

3.8 Data treatment

All results are expressed on a fresh weight or lipid weight basis. PCDD/PCDFs and PCBs results are all reported on an upper bound basis. Mean reported values for PCDD/PCDFs and PCBs were calculated by summation of the total divided by the number of samples. Mean reported values for metallic elements were calculated by summation of the total of detected values (all non-detected values were excluded) divided by the number of samples with detectable results.

4. Results

4.1 Biometric data

The mean fork length of YTKF was 58 cm (range 49-75) and for Mulloway was 47 cm (range 44-56). The mean weight (gilled and gutted) of YTKF was 3.1 kg (range 1.6-6.1) and for Mulloway was 1.3 kg (range 1.1-2.3).

4.2 Analytical results

PCDD/PCDFs and PCBs were found in all samples of YTKF, Mulloway and manufactured aquaculture feeds (Table 1). PCDD/PCDFs contributed a greater proportion to the TEQ in some samples of aquaculture feed than was observed in any of the fish samples (Figure 2). Dioxin-like PCBs represented the greatest contributor to the total TEQ reported in fish samples (Figure 2). The mean concentration of PCDD/PCDFs and dioxin-like PCBs in the YTKF was 2.6 pg TEQ/g (range 1.4-3.5), while the Mulloway had a mean concentration of 0.67

pg TEQ/g (range 0.57-0.76) (Table 1). The manufactured aquaculture feed samples had a mean concentration of PCDD/PCDFs and dioxin-like PCBs of 2.0 pg TEQ/g (range 0.29-4.6). The mean lipid concentration of the YTKF was 10 % (range 1.9-18), while the Mulloway was 2.7 % (range 1.8-5.0). The mean lipid concentration of the manufactured aquaculture feed samples was 19% (range 15-21) (Table 1).

Cadmium was not detected in any fish sample in this study (Table 2). It was, however, detected in all manufactured aquaculture feed samples at a mean concentration of 0.21 mg/kg (range 0.16-0.36). Mercury was detected at low levels in all fish samples. The mean mercury concentration in YTKF was 0.03 mg/kg (range 0.02-0.05) and in Mulloway it was 0.023 mg/kg (range 0.02-0.04). All of the manufactured aquaculture feed samples contained mercury at a mean concentration of 0.04 mg/kg (range 0.01-0.13). Lead was only detected in a single sample of YTKF at 0.03 mg/kg. All of the manufactured aquaculture feed samples contained lead at a mean concentration of 0.23 mg/kg (range 0.11-0.29). Copper was present at levels <1 mg/kg in all YTKF and Mulloway samples, while in feed it was present in all samples at levels approximately 10-fold higher. Manufactured feed, being a shelf stable product, has a reduced moisture content compared to fresh fish; hence levels in feed samples may appear inflated due to moisture content differences

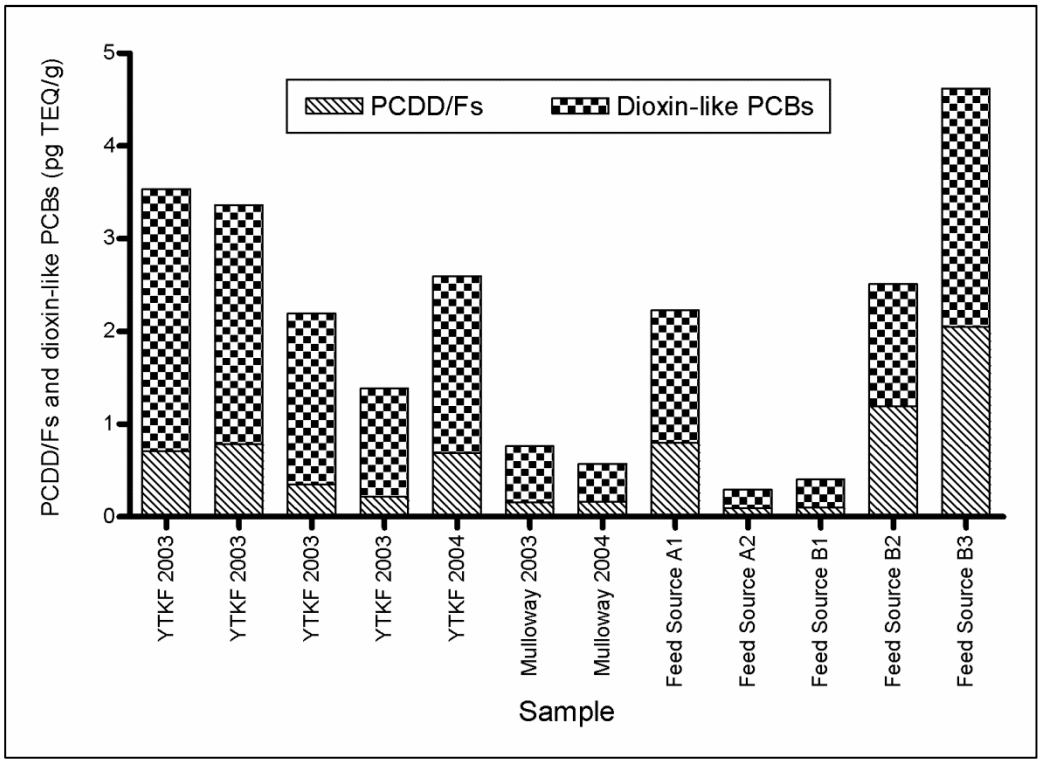

Figure 2. PCDD/PCDFs and dioxin-like PCBs concentration found in Australian farmed Yellowtail Kingfish (*Seriola lalandi*), Mulloway (*Argyrosomus japonicus*) and manufactured aquaculture feeds (manufacturing source identified by A or B). All results fresh weight basis.

There were no detectable levels of any pesticide in any sample of YTKF, Mulloway or aquaculture feed. Similarly, no antimicrobial compounds were detected in any YTKF or Mulloway samples (feed samples were not analysed for antimicrobials as a suitable validated laboratory method was not available). The European Union maxium content values for feeds (Tables 1 and 2) only apply to feed with a moisture content of 12%. In the present study the moisture content of feed samples was not determined.

Table 1. Mean PCDD/PCDFs and PCB concentrations in Australian farmed Yellowtail Kingfish, Mulloway and manufactured aquaculture feeds.

Sample	***n***	**Lipid**	**PCDD/PCDFs**		**Dioxin-like PCBs**		**PCBs[1]**		**PCDD/PCDFs & dioxin-like PCBs**	
Units	N/A	%	pg TEQ/g		pg TEQ/g		ng/g		pg TEQ/g	
Reporting basis[1]	N/A	N/A	f.w.	l.w.	f.w.	l.w.	f.w.	l.w.	f.w.	l.w.
Australian ML	N/A	N/A	Not set	Not set	Not set	Not set	500	Not set	Not set	Not set
Japanese MAL	N/A	N/A	Not set	Not set	Not set	Not set	500	Not set	Not set	Not set
European Union ML	N/A	N/A	4	Not set	Not set	Not set	Not set	Not set	8	Not set
Yellowtail Kingfish	5	10 (1.9-18)	0.55 (0.22-0.78)	4.8 (2.9-7.8)	2.1 (1.2-2.8)	18 (15-22)	21 (8.6-29)	183 (116-326)	2.6 (1.4-3.5)	23 (18-29)
Mulloway	2	2.7 (1.8-5.0)	0.16 (0.16-0.16)	4.7 (2.8-6.6)	0.51 (0.41-0.61)	18 (11-16)	5.4 (4.7-6.0)	162 (84-240)	0.67 (0.57-0.76)	18 (13-23)
European Union MC[2]	N/A	N/A	2.25	Not set	Not set	Not set	Not set	Not set	7.0	Not set
Aquaculture feeds	5	19 (15-21)	0.85 (0.096-2.1)	4.5 (0.49-10)	1.2 (0.30-2.6)	6.1 (1.1-12)	18 (1.5-42)	93 (7.8-204)	2.0 (0.29-4.6)	11 (1.6-22

[1]PCBs: Summation of 45 congeners including non-ortho and mono-ortho PCBs.

[2]The European Union maxium content values (EU Directive 2002/32/EC) apply to feed with a moisture content of 12%. In the present study the moisture content of feed samples was not determined.

f.w.: fresh weight

l.w.: lipid weight

Figures in parentheses indicate the range.

MAL: maximum allowable level (equivalent to maximum level)

MC: maximum content relative to a feed with a moisture content of 12 %

ML: maximum level

n: number of samples

N/A: not applicable

Table 2. Mean concentrations of metals and metalloids (f.w. basis) in Australian farmed Yellowtail Kingfish, Mulloway and manufactured aquaculture feed.

Sample	*n*	Metals (mg/kg)					Metalloids (mg/kg)	
		Cadmium (Cd)	Copper (Cu)	Lead (Pb)	Mercury (Hg)	Zinc (Zn)	Arsenic (As)	Selenium (Se)
LOR	N/A	0.01	0.01	0.01	0.01	0.01	0.02	0.05
Australian ML[3]	N/A	Not set	Not set	0.5	0.5	Not set	Not set[4]	Not set
Japanese MAL	N/A	Not set	Not set	Not set	Exempt	Not set	Not set	Not set
European Union ML	N/A	0.05	Not set	0.3	0.5	Not set	Not set	Not set
Yellowtail Kingfish	27	<0.01[2]	0.6 (0.5-0.7)	0.01[1] (<0.01-0.03)	0.03 (0.02-0.05)	3.1 (2.4-4.8)	0.9 (0.3-1.4)	0.2 (0.1-0.4)
Mulloway	6	<0.01[2]	0.34 (0.26-0.46)	<0.01[2]	0.023 (0.02-0.04)	3.2 (2.3-4.9)	0.5 (0.3-0.7)	0.14 (0.09-0.35)
European Union MC[5]	N/A	1	Not set	5	0.2	Not set	10	Not set
Aquaculture feeds	5	0.21 (0.16-0.36)	8.3 (7.4-9.3)	0.23 (0.11-0.29)	0.04 (0.01-0.13)	164 (120-200)	1.7 (0.95-3.7)	1.4 (0.85-1.7)

[1]There was only one sample of YTKF with a quantifiable level of Pb present.

[2]Results were all less than the Limit of Reporting (LOR).

[3]There are no Australian Maximum Levels (MLs) set for metals or metalloids in aquaculture feeds.

[4]The ML for arsenic in fish applies to inorganic arsenic (2 mg/kg). This study measured total arsenic.

[5]The European Union maxium content values (EU Directive 2002/32/EC) apply to feed with a moisture content of 12%. In the present study the moisture content of feed samples was not determined.

ML: maximum level

MAL: maximum allowable level

MC: maximum content relative to a feed with a moisture content of 12 %

LOR: limit of reporting

Figures in parentheses indicate the range

n: number of samples

N/A: not applicable

f.w.: fresh weight basis

5. Discussion

Seafood is an important food for many people around the world including Australia; public health benefits of regular seafood consumption are being confirmed and quantified in various studies worldwide (Domingo et al., 2007c). In this study results were compared against regulatory standards where set by Australia (set by Food Standards Australia New Zealand), Japan (set by the Japanese Ministry of Health, Labour and Welfare) and European Union (set by the European Commission) authorities (Tables 1, 2, 3 and 4).

Levels of organic contaminants such as PCBs in YTKF were on average 4% (range 2-6) of the Australian Maximum Level (ML), while in Mulloway they were approximately one-quarter of the values for YTKF (Table 1). Levels of PCDD/PCDFs were on average 14% (range 6-20) of the European Union (EU) ML, while for Mulloway they were on average 4% of the EU ML. PCDD/PCDFs and dioxin-like PCBs in YTKF when compared to the EU ML, were on average 33% (range 18-44), while for Mulloway they were 8% (range7-10) (Table 1). The contribution of the PCDD/PCDFs to the total TEQ value in YTKF was 21%, while for the dioxin-like PCBs it was 79%. In Mulloway the contribution of the PCDD/PCDFs was 24% to the total TEQ value, while the dioxin-like PCBs contribution was 76%. The reasons for the differences reported in the the levels of PCDD/PCDFs and dioxin-like PCBs between the two species may potentially be related to the physiology of each species thus potentially having implications for risk managers. Controlled feeding studies could be undertaken to investigate these preliminary results, coupled with characterisation of

the distribution of these and other residues and contaminants in these fish would help to inform future risk profile studies. Information on congener profiles in diet samples and harvested fish Total PCB concentration have been reported in Japanese Yellowtail (*Seriola quinqueradiata*) at up to 0.95 mg/kg (the present study found total PCBs up to 0.029 mg/kg – Table 1) (Watanabe et al., 1979). Other Japanese studies have reported the presence of PCBs and other organochlorine contaminants in other Seriola species (Takamiya et al., 1992, Ueno et al., 2002).

Levels of metallic elements such as mercury in YTKF, when compared to Australian and European Union standards, were on average 6% (range 4-10), while in Mulloway they were on average 5% (range 4-7) (Table 2). A single Australian study reported mercury in wild caught YTKF from New South Wales (NSW) coastal waters (n=123) in 1977 and 1978 (Chvojka, 1988). Chovjka found mean total mercury concentration in white axial head muscle tissue at 0.15 mg/kg (range 0.01-1.13). The present study found levels approximately one-fifth of these (Table 2). The reasons for this difference could be related to the lipid content of the farmed YTKF being higher than that of their wild counterparts, the differences in diet, the age of fish and the portion tested. While Japanese farmed Yellowtail (*Seriola quinqueradiata*) fed on a sardine diet was reported to have had a mean mercury concentration of 0.05 mg/kg in dorsal muscle (equal to the maximum mercury concentration found in the present study); Yellowtail fed on a mackerel diet was reported to have had a mean mercury concentration of 0.09 mg/kg in dorsal muscle (Takeda and Ueda 1979).

Cadmium was not detected in any fish sample and lead was detected in a single YTKF sample at 6% of the Australian ML (Table 2). Lead does not appear to accumulate in other commercially farmed species of finfish in the Spencer Gulf such as Southern Bluefin Tuna (*Thunnus maccoyii*) (Padula et al., 2008). Copper has a number of industrial uses in the marine environment e.g. ship hull antifoulant treatments. At higher levels copper may act as an immunosuppressant, increasing susceptibility to parasitic infection (Zelikoff, 1993; Fernandes et al., 2009). Copper was not detected in either YTKF or Mulloway (Table 2). Zinc and selenium were present in all fish samples (Table 2).

Feed samples had detectable levels of several contaminants including dioxins, PCBs, cadmium, lead and mercury (Tables 1 and 2). Dietary uptake of these contaminants is affected by a number of factors including metabolism of these compounds, polarity of compounds, duration and magnitude of exposure and growth state of fish (Jacobs et al., 2002; Lundebye et al., 2004). The reason why contaminants such as cadmium and lead (found in feed) did not appear to be retained in the edible tissue of these species could be due to the homogeneity of the feed samples taken or a rapid turnover of individual feed bags, fish physiology, or possibly that fish were consuming other wild fish living in the sea-cages. The origin of these contaminants in manufactured feed may be from several raw ingredients including fish oil, binder clays, fish meal and other additives. Marine origin fish oils used in manufactured aquaculture feeds may be inadvertently contaminated with lipid-soluble pollutants. Vegetable oils and other lipid sources of

animal origin have been identified as suitable for inclusion in some aquaculture feeds as alternatives to using fish oils in aquaculture diets (Caballero et al., 2002; Jacobs et al., 2002). Manufactured aquaculture feed ingredients are sourced from multiple origins depending on availability, quality, cost and nutritional criteria. Pre-purchase screening of diet inclusion ingredients for manufactured aquaculture feeds may provide early warning of potential food safety issues (Kan and Meijer, 2007).

A number of issues are of significance to the Australian industry in gaining market access. When accessing international markets, exporters need to be aware of the technical basis of the importing country's national residue and contaminant standards. This requirement may see testing undertaken at frontier border inspection stations using methods of sampling and analyses not employed in the exporting country. Limited availability of suitable laboratory capability and access to validated laboratory methods may hinder seafood exporters in gaining market access to some countries.

Port of entry inspection programs in international markets operate to fulfil local domestic food standards. However the technical basis of these standards, even if numerically identical, are seldom technically equivalent due to reasons which include methods of sampling, laboratory methodology, residue and contaminant definitions and reporting conventions (Huggett et al., 1998). In addition revisions to standards often occur to reflect current or emerging hazards (Abbott et al., 2003). One example is the lower, medium and upper bound

reporting of dioxins and PCBs, with the European Union using upper bound, Japan using lower bound while Australia uses middle bound results reporting (Müller et al., 2004). Further complicating this is the use of different TEF values, making numerical comparisons of results difficult (results can be inflated or deflated depending on which approach is taken); for trade purposes this information is not always made clear when test results are given (Van den Berg et al., 1998; Van den Berg et al., 2006).

Potential sources of contaminants of such as pesticide residues in farmed fish may arise from run-off from terrestrial crop application into the marine environment, leading to contamination of fish species which are used as manufactured feed ingredients (Campos et al., 2005; Kelly et al., 2008). In developing countries, from where some manufactured feed ingredients may be sourced, pesticides such as DDT may still be in active use to meet domestic economic and public health needs, despite de-registration of these same products in developed countries (Attaran et al., 2000; Ecobichon, 2001; Connell et al., 2002; Kan and Meijer, 2007).

Veterinary medicine residues in aquaculture fish can occur following treatment of aquatic diseases where the withholding period is not fully observed. However, some cases may arise from sources in the marine environment, such as sewage treatment plant effluent (Katae et al., 1980; Hirsch et al., 1999; Kenkyo, 2000). Upon ingestion, metabolism and biotransformation of veterinary medicines may occur for example, hydroxylation of the anthelmintic praziquantel (Tubbs et al., 2008).

Technical information for exporters of YTKF and Mulloway on sampling methods, residue and contaminant definitions, portion to which the standard applies, laboratory methods of analysis and reporting conventions in importing countries are not always available. This information would allow exporters to make independent evaluations of equivalence on each country's port of entry requirements against those required for export from Australia and inform pre-export testing programs.

All Australian farmed YTKF and Mulloway in this study complied with residue and contaminant standards (where set) in Australia, Japan and the European Union for the tests reported in the present study. Further work is required to investigate accumulation of PCDD/PCDFs, PCBs and key metallic elements in aquaculture species. The results from this study will assist in informing future risk vs. benefit and risk assessment studies. Exporters of aquaculture fish need to be aware of technical differences in the interpretation and application of port of entry testing requirements and standards when assessing new markets.

6. Acknowledgements

This research was financially supported by a grant from the Australian Government Fisheries Research and Development Corporation (FRDC). We would like to thank Dr Andreas Kiermeier and Dr Cath McLeod of the South Australian Research and Development Institute for reviewing the manuscript and providing helpful comments. We would also like to thank Mr Phil Bridgen of AsureQuality (formerly AgriQuality) for technical assistance with dioxin and PCB analyses, Ms Hao Nguyen of the Australian Government National Measurement Institute (NMI) for technical assistance with antimicrobial analyses, Dr Honway Louie (retired), Dr Michael Wu and Mr Paul Adorno of NMI for technical assistance with metallic analyses, Mr Daniel Slee of NMI for technical assistance with pesticide analyses and Mr Geoffrey Thurtell of NMI for administrative assistance with data reports.

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CHAPTER FIVE

Manuscript in preparation

Padula, D.J.; Daughtry, B.J.; Flint, R.; Nowak, B.F. Mercury content of wild Australian Southern Bluefin Tuna (*Thunnus maccoyii*) destined for aquaculture fattening: Comparison with bluefin tunas in the Japanese market. In preparation.

1. Introduction

Marine predatory fish such as tuna species play an important role in the diet and traditional food culture of many countries (Bestor, 2001). The preparation of dishes such as sushi and sashimi, of national culinary importance in Japan, could not be achieved without the inclusion of tuna (De Silva and Yamao, 2006). The tunas which are supplied to the Japanese market may be wild or farmed in origin, from worldwide sources (Sonu, 2008). Australian Southern Bluefin Tuna (SBT) (*Thunnus maccoyii*) (Castelnau, 1872) destined for Japan are farmed from wild capture SBT in sea-cage farms offshore at Port Lincoln in South Australia (SA). Juvenile SBT are captured by purse seining from the Great Australian Bight between December and March each year (Australian Southern Bluefin Tuna Industry Association, 2011). The SBT are fattened for a period of several months in near-shore sea-cages at Port Lincoln before harvest for export to the premium fish markets (since 1995) of Japan, where they are locally known as Minami Maguro (Sonu, 2008; Australian Southern Bluefin Tuna Industry Association, 2011).

Mercury is widely distributed in the marine environment, principally from volcanic eruptions (Pyle and Mather, 2003). In the marine environment bacteria on the ocean's floors convert this mercury other chemical forms which are accumulated by marine organism which subsequently become food for fish (King et al, 2000; Kerin et al, 2006). Thus mercury is transferred from the marine environment to consumers of seafood products; public health risks of mercury from eating fish, including subtle neurological and developmental effects on

children, have been studied extensively (Brieger and Rieders, 1959; Balshaw et al, 2007). During the 1960s and 1970s large accidental poisonings due to mercury compounds inadvertently present in fish and grain occurred in Japan and Iraq (Clarkson et al, 1976; Weiss, 2007).

Fish contains a range of nutrients of public health significance including minerals, quality protein, fatty acids, vitamins and other nutritional components (Zhang et al, 1999; Mozaffarian and Rimm, 2006; Diaz and Hu, 2009). Some of these, such as the mineral selenium, have a synergistic harm-minimising effect on toxic metals such as mercury when consumed in the diet (Potter and Matrone, 1974; Ohi et al, 1976; Itano et al, 1977; Berlin, 1978; Pelletier, 1985; Cuvin-Aralar and Furness, 1991; Yoshia, 2008; Raymond and Ralston, 2009). Complex biochemical reactions involving biological thiols such as cysteine components present in proteins occur between mercury and selenium compounds when ingested, to protect against neurotoxicity (Potter and Matrone, 1974; Shimojo et al, 1975; Pařízek, 1990; Watanabe, 2002; Yoshida et al, 2011). Both wild and farmed Australian SBT contain appreciable levels of selenium (Padula et al, 2008). Other key minerals present in tunas, such as iodine, play a key role in cognitive function and psychomotor development (Vorodimova, 1980). It is important to establish which chemical form of mercury is present to fully evaluate public health risk in foods of marine origin, methylmercury being the most toxic form for causing neurological damage, particularly to the unborn child (Amano, 1976; Harris, 2003; Codex Committee on Food Additives and Contaminants, 2005; Honda et al, 2006; Pikholz and

Simmons, 2006; Itano, 2007; New Zealand Food Safety Authority, 2008; Satoh, 2008; Pikholz and Simmons, 2010).

Foods which contain mercury, such as fish, are regulated by national competent authorities using regulatory limits (Shimshack and Ward, 2010; Lando and Zhang, 2011). Supporting these regulatory standards, public consumption advisories may also be issued to protect sensitive population groups such as pregnant women and children through specific dietary advice (Food Standards Australia New Zealand, 2004a).

In terms of population exposure to mercury, in Japan, seafood products account for 90% of the mercury exposure through the national diet (Nakagawa, et al., 1997). The technical basis of this Japanese dietary exposure assessment is based on a 50 kg adult body weight; in contrast Australia relies on a 67 kg adult body weight to inform risk assessment studies (Aoki, 1970; Shishido and Suzuki, 1974; Suzuki et al, 1976; Nishikawa and Toyokawa, 1998; Zhang, 2009). Whether these figures are reflective of present day body weight ranges is questionable; this approach however does provide a higher level of protection and compensation for other assumptions made in exposure assessments.

The Provisional Tolerable Weekly Intake (PTWI) for methylmercury in 2003 was reduced from 3.3 μg/kg body weight (b.w.) to 1.6 μg/kg b.w. by the Joint Expert Committee on Food Additives (JECFA) of the Food and Agriculture Organisation of the United Nations (FAO) and the World Health Organisation (WHO) (Joint FAO/WHO Expert Committee on Food Additives, 2003). This lower value was

reconfirmed in 2006 (Joint FAO/WHO Expert Committee on Food Additives, 2006).

However, methylmercury has been shown to be unstable in frozen stored fish samples; significant losses of more than half have been reported following storage from as little as 15 days at -25°C (Devai et al, 2001). Methylmercury is subject to change in its chemical form due to the action of thermophilic sulphate and or iron reducing bacteria naturally present in the marine environment (King et al, 2000; Kerin et al, 2006). Its measurement in fish samples may not be a reliable indicator of contamination. Total mercury concentration is unchanged by storage conditions in biological materials, hence the present study selected to analyse only total mercury content (De Boer and Smedes, 1997).

Tuna species of economic importance in Australia and Japan include SBT, Atlantic Bluefin Tuna (ATB) (*Thunnus thynnus*) (Linnaeus, 1758) and Pacific Bluefin Tuna (PBT) (*Thunnus orientalis*) (Temminck and Schlegel, 1844; Collette and Naven, 1983). SBT are commercially farmed in South Australia, through aquaculture sea-cage farming over a period of several months prior to harvest and export to Japan. In the 2008/2009 period 8,786 T (gilled, gutted and tail off) of farmed Australian SBT were produced, with a total value of $AU166 M (Ferris and Morison, 2010; Pham, 2010). Principally, these farmed SBT were exported to the premium fish markets of Japan, including the Tokyo Metropolitan Central Wholesale Market (Tsuijiki market) (Australian Southern Bluefin Tuna Industry Association, 2011).

The total mercury content of Australian farmed SBT has been reported previously; however, no comparative information has been published for Australian wild SBT destined for aquaculture fattening for the Japanese market (Balshaw et al, 2008a; Balshaw et al, 2008b; Padula et al, 2008).

2 Aim of study

The primary aim of this study was to generate occurrence data for total mercury in wild Australian SBT destined for aquaculture fattening and to assess results against comparative bluefin tunas available in the Japanese market. A secondary aim was to evaluate results from the present study against Australian food regulatory standards and public health consumption advisories, especially in light of differing consumer body weights used by Australia and Japan for undertaking exposure assessments.

3 Methods

3.1 Sample collection

SBT (n=100) were collected between January and April 2005 by Australian Fisheries Management Authority (AFMA) staff across all tow-ins for the 2005 aquaculture grow-out season. SBT were individually poled during purse seining. From each individual SBT a one kg caudal sample containing dorsal and ventral muscle tissue was taken. Samples were stored frozen on board of each boat at -20°C in individual sealed plastic bags containing a waterproof identification tag

until return to shore. Storage temperatures less than -5°C have been shown to have no influence on total mercury content in biological materials (De Boer and Smedes, 1997). The fork length of each individual tuna (caught by purse seining) was recorded by an Australian Fisheries Management Authority (AFMA) staff member on board of the tuna boat with a ruler board specifically designed for measurement of tuna fish, but it was not possible to collect fish weights due to fish being taken at sea on operating commercial boats.

3.2 Sample processing and storage

Each sample was defrosted at 4°C overnight. The skin, bones and red muscle were removed from each sample because these are considered inedible and their removal allowed the preparation of a consistent, homogenous analytical sample (Lichton, 1992; Krejčová et al, 2008). Any thawing juices were added to the food processor prior to homogenising the sample. Samples were individually homogenised in a stainless steel HOBART™ food processor. All equipment, tables, benches, knives, the food processor and its components were cleaned with DECON90 (Bacto Laboratories, Australia) laboratory detergent and double rinsed with mains tap water before and after each sample was processed. All samples were double bagged to protect sample integrity and stored at -80°C for 30 days until despatched to a laboratory for analysis. Samples were couriered to the laboratory in a sealed foam box in which several freezer gel packs were placed. The laboratory stored samples initially at -20°C upon arrival until documentation checks were undertaken and samples logged in the laboratory information management system. Following these checks,

samples were defrosted at 4°C for 24 hours prior to the commencement of analysis; all thawing juices were included in the analytical sample.

3.3 Mercury determination

Mercury determinations were undertaken by an Australian laboratory accredited by the National Association of Testing Authorities (NATA) to the requirements of ISO/IEC 17025 (1999). The method used for analysis of total mercury was based on USEPA Method 3050 "Acid digestion of sediments, sludges and soil", and from the preparation stage of USEPA 200.7 Revision 5.0 "Trace elements in water, solids and bio-solids by Inductively Coupled Plasma-Atomic Emission Spectrometry".Mercury was extracted from each SBT sample (1 g) by digestion on a heating block (90°C for 90 minutes) with concentrated nitric acid (Merck, analytical grade) and hydrogen peroxide (Merck, analytical grade) to which stannous chloride (Merck, analytical grade) in 7% hydrochloric acid (Merck, analytical grade) was added to stabilize the mercury. Sample analysis was performed via Cold Vapor Atomic Absorption Spectroscopy (CETAC M-7500 Mercury Analyser, United States) at an absorbance of 253.7 nm. Blanks and mercury standards (Choice Analytical) 10 μg/L, 100 μg/L, 1000 μg/L were used with each batch of samples. The limit of reporting (LOR) was 0.01 mg/kg (f.w. basis).

3.4 Quality assurance and quality control

The contract laboratory ran every 10^{th} sample in duplicate and every 20^{th} sample was spiked. The spiked sample recoveries were on average 83% (range 80-87). Duplicates had a relative percent deviation on

average of 9% (range 3-20). Blanks were all less than the Limit of Reporting (LOR) of 0.01 mg/kg.

3.5 Dietary exposure assessment

A deterministic exposure assessment to evaluate public health risk of consumption of SBT is reported in Table 1. This was performed following the methodology of Food Standards Australia New Zealand (Food Standards Australia New Zealand, 2004a, Food Standards Australia New Zealand, 2011b; Food Standards Australia New Zealand. 2011c). This was based on the assumption that Australian women and women planning pregnancy had a body weight of 66 kg, the Australian general population a body weight of 67 kg and Australian children (up to six years of age) had a body weight of 19 kg. A serving size of 150 g was used for all adults and for children a value of 75 g was used. A Permissible Tolerable Weekly Intake (PTWI) of 1.6 μg/kg body weight per week was used for Australian pregnant women and women planning pregnancy while for all other groups a PTWI of 3.3 μg/kg body weight per week was used. It was assumed that all mercury was present as methylmercury in calculations, with non-seafood items contributing 0.09 % of methylmercury to the diet for all adults and 0.01 % for children.

Food Standards Australia New Zealand undertakes its risk assessments based on total mercury measurements (based on median values), assuming that all mercury present in seafood samples is present as methylmercury. This approach assumes that people only eat one type of fish, with a serving being 150 g for the adult population and 75 g for

children of 6 years or older (Food Standards Australia New Zealand, 2004b). This approach may overstate the potential public health risk of mercury in some consumers, but offers the greatest degree of protection to vulnerable population groups. Comparisons between Australian and Japanese public mercury consumption advisories must take into consideration differences in consumers' body weight (50 kg vs. 67 kg) relied upon for calculation purposes and the portion of tuna which is consumed (including effects of cooking) (Arima and Umemoto, 1976). The most recent Australian body weight data (self reported) reports adult men (all ages) with a mean weight of 84 kg and adult women (all ages) with a mean weight of 68 kg (Australian Bureau of Statistics, 2008). The effect of this is a more conservative outcome which provides a higher level of public health protection to Australian consumers.

3.6 Identification of published mercury data and standards for comparative purposes

For comparative purposes, published mercury data from Japanese port of entry testing and Japanese Government Agencies testing programs were identified and collated. Mercury data were sourced from public websites of the Japanese Ministry of Agriculture, Forestry and Fisheries (MAFF), Japanese Fisheries Research Agency (FRA) and Japanese Ministry of Health, Labour and Welfare (MHLW) (Ministry of Health, Labour and Welfare, 2005a; Ministry of Agriculture, Forestry and Fisheries, 2011). Japanese bluefin tunas total mercury content data were available for the years 2002-2004; all Japanese SBT mercury content data were from 2003 (exact sample collection date, location and water temperature information was not available). All wild Australian SBT

data reported from the present study is from 2004. Japanese language reports were translated into English and tabulated (performed by Mr Tim Tanaka, Food SA office, Osaka, Japan).

Mercury standards that apply to tuna domestically and internationally have been tabulated and summarised in Table 2 (United Nations Environment Program, 2002).

3.7 Statistical treatment of data

Statistical analysis and preparation of figures were performed using R software (R Development Core Team, 2011). Mean reported mercury concentrations from published sources by species and origin were calculated assuming non-detected values were equal to the Limit of Detection (LOD). All results were standardised to mg/kg units (fresh weight basis). Mercury regulatory standards were standardised to mg/kg units (Table 2). For the wild caught Australian SBT total mercury data fom the present study, an Analysis of Variance (ANOVA) was used to test for differences in $\log_{10}$ mercury due to fork length (cm). The fitted linear equation is

$$\text{Log}_{10}(\text{Total Mercury}) = -0.5305 + 0.0013 \times \text{Fork Length (cm)}$$

This equation applies only to data from the present study (Origin=1). Log_{10} was used because the data showed deviations from normality. A significance level of 5% was used. The assumptions for fitting a linear model were checked using plots (not shown). The standard deviation of the residuals is 0.1077. For all other data only the Origin was examined

due to sample size, insufficient information on collection methods, analytical methods, catch or farming location, water temperature and treatment of data. It is speculated that fork length measurements may have been collected differently in each of the cited studies (flat measurement versus folded measurement and or use of total body length); hence fork length was not used in the comparisons of data from the present study with Japanese reported data.

4 Results

Total mercury (fresh weight basis) was present in all Australian wild SBT samples with the mean total mercury concentration being 0.43 mg/kg (range 0.24-0.72) (Figure 1). In Figure 1, total mercury content of Australian and Japanese tuna (wild and farmed) species from the present study and from Japanese Government sources is presented (Ministry of Agriculture, Forestry and Fisheries, 2011; Ministry of Health, Labour and Welfare, 2005a). Japanese data include results for different edible portions in each tuna species (but not more than one filleted cut per individual tuna was reported) based on Japanese filleting including the o-toro, chu-toro and akami.

There was a significant effect on $\log_{10}$ mercury content due to origin ($p<0.001$), fork length ($p<0.001$) and the interaction between origin and fork length ($p<0.001$). This means that the relationship between $\log_{10}$ mercury content and fork length varies depending on the origin. For example, $\log_{10}$ mercury content increases as fork length increased for Australian wild caught SBT from the present study, whereas $\log_{10}$ mercury content decreased as fork length increased for Japanese wild

caught SBT. Excluding fork length from the analysis, there was no significant difference in mercury content between Japanese caught wild SBT and Australian wild caught SBT ($p<0.001$). The mercury content of Japanese tested Australian farmed SBT were significantly lower than the mercury content of Japanese caught wild SBT and Australian caught wild SBT. The mercury content for Japanese caught wild ABT and Japanese farmed PBT were both significantly higher than the mercury content in wild Australian SBT, Japanese wild SBT and Japanese tested Australian farmed SBT. The reasons for this may be due to water temperature and catch location effects as described in farmed Japanese PBT (Ando et al, 2011). Other possible reasons could include species differences and effects of migration.

The dietary exposure assessment found that Australian pregnant women and women planning pregnancy can consume 1 serving (150 g) of Australian wild SBT per week, while the Australian general population can consume 3 serves (150 g each) per week and Australian children (up to six years old) can consume 1 serve (150 g) per week without reaching the Permissible Tolerable Weekly Intake (PTWI) (Table 1).

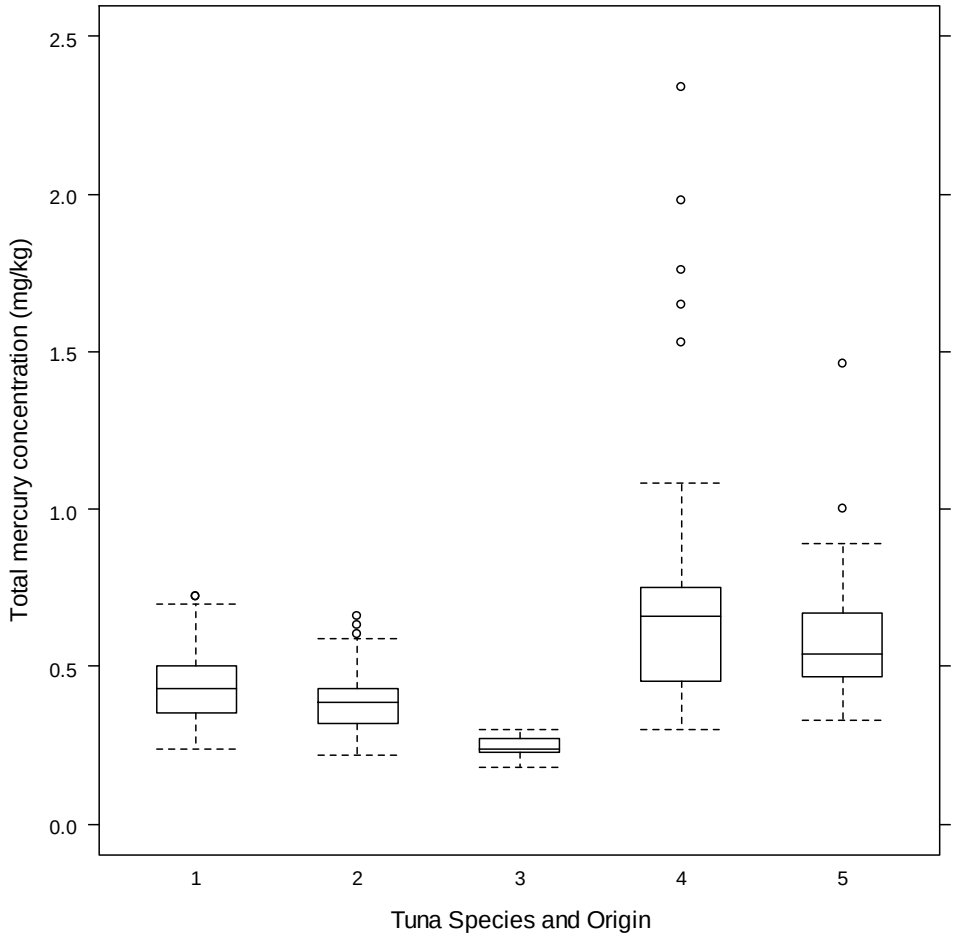

Figure 1. Total mercury content (fresh weight basis) of wild Australian Southern Bluefin Tuna (*Thunnus maccoyii*) (the present study) and related tuna species farmed or caught in Japan. Tuna species and origin: 1. Australian wild Southern Bluefin Tuna (the present study) 2. Wild Japanese caught Southern Bluefin Tuna 3. Australian farmed Southern Bluefin Tuna (Japanese port of entry testing) 4. Wild Japanese caught Atlantic Bluefin Tuna (*Thunnus thynnus*) 5. Japanese farmed Pacific Bluefin Tuna (*Thunnus orientalis*).

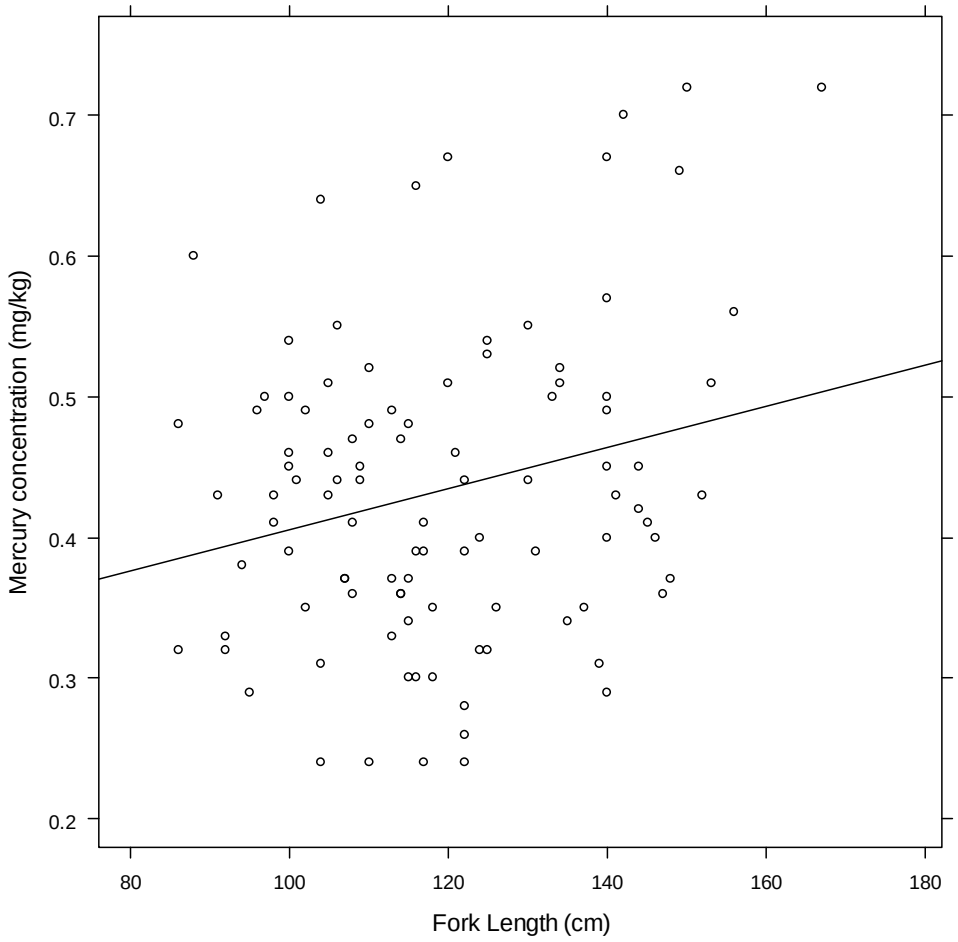

Figure 2. Total mercury concentration (fresh weight basis) of wild Australian Southern Bluefin Tuna (*Thunnus maccoyii*) vs. fork length.

The mean fork length of wild SBT (n=100) in the present study was 119 cm (range 86-167); that of Japanese wild SBT (n=42 collected in 2003) was 96 cm (range 80-140); the mean fork length of Japanese tested Australian farmed SBT (n=30 collected in 2003) was 119 cm (range 87-167), the mean fork length of Japanese wild caught ABT was 138 cm (range 58-214 collected in 2003), while the mean fork length of Japanese

farmed PBT (n=30 collected over the period 2002-2004) was 104 cm (Figure 2).

Table 1. Dietary exposure assessment for methylmercury from consumption of Australian wild Southern Bluefin Tuna.

Exposure assessment inputs	Australian pregnant women and women planning pregnancy	Australian general population	Australian children (up to 6 years)
Permissible tolerable weekly intake of methylmercury	1.6 μg/kg body weight/week	3.3 μg/kg body weight/week	3.3 μg/kg body weight/week
Body weight	66 kg	67 kg	19 kg
Serving size	150 g	150 g	75 g
Total permitted methylmercury intake per week	105.6 μg/week	221.1 μg/week	62.7 μg/week
Estimated methylmercury intake from non-seafood items in the diet (main source spices)	0.94 μg/week (0.09 % of total methylmercury exposure from all foods)	1.14 μg/week (0.09 % of total methylmercury exposure from all foods)	3.10 μg/week (0.01 % of total methylmercury exposure from all foods)
Amount of methylmercury that can be consumed from fish sources	= 104.6-0.94 μg/week = 104.66 μg/week	= 221.1-1.14 μg/week = 219.96 μg/week	= 62.7-3.10 μg/week = 59.60 μg/week
Maximum amount of wild Australian Southern Bluefin Tuna that can be consumed per week[1]	= 104.66 μg/week ÷ 430 μg/kg = 243 g SBT/week = 1.6 serves SBT/week	= 219.96 μg/week ÷ 430 μg/kg = 512 g SBT/week = 3.4 serves SBT/week	= 59.60 μg/week ÷ 430 μg/kg = 139 g SBT/week = 1.8 serves SBT/week

Exposure assessment inputs	Australian pregnant women and women planning pregnancy	Australian general population	Australian children (up to 6 years)
	1 serve SBT/week	3 serves SBT/week	1 serve SBT/week

[1]Assuming median total mercury concentration of 430[1] μg/kg and that all mercury were present as methylmercury

Table 2. Summary of international mercury standards which apply to bluefin tunas (United Nations Environment Program, 2002).

Country	Portion to which the standard applies	Standard (mg/kg)	Basis of standard	Note:s
Australia	Edible content as ordinarily consumed	1	Total mercury	Compliant if mean value of 10 or more sample units is ≤ 1 mg/kg with no individual sample unit > 1.5 mg/kg
Canada	Not specified	0.5	Total mercury	
Codex Alimentarius Commission	Not specified	1	Methylmercury	Guideline value only
European Union	Not specified	1	Total mercury	
Hong Kong Special Administrative Region of the People's Republic of China	Not specified	0.5	Total mercury	
Japan	N/A	Not set	N/A	All tuna species are exempt from the Food Sanitation Law
Kingdom of Norway	Not specified	1	Total mercury	
Kingdom of Thailand	Not specified	0.5	Total mercury	
Malaysia	Not specified	0.5	Methylmercury	

Country	**Portion to which the standard applies**	**Standard (mg/kg)**	**Basis of standard**	**Note:s**
New Zealand	Edible content as ordinarily consumed	1	Total mercury	Compliant if mean value of 10 or more sample units is ≤ 1 mg/kg with no individual sample unit > 1.5 mg/kg
People's republic of China	Not specified	1	Methylmercury	
Republic of Cuba	Not specified	1	Total mercury	
Republic of India	Not specified	0.5	Total mercury	
Republic of Korea	Not specified	0.5	Total mercury	
Republic of Mauritius	Not specified	1	Total mercury	Action level.
Republic of Senegal	Not specified	0.7	Total mercury	Threshold limit
Republic of Singapore	Not specified	0.5	Total mercury	
Republic of Zimbabwe	Not specified	1	Methylmercury	
Socialist Republic of Vietnam	Not specified	1	Total mercury	
Taiwan	Edible portion	2	Methylmercury	
United States of America	Not specified	1	Methylmercury	

5 Discussion

The mean mercury concentration of Australian wild SBT found in the present study was less than half of the Australian regulatory standard (Food Standards Australia New Zealand. 2011a). A dietary exposure assessment found that Australian pregnant women and Australian women planning pregnancy could consume one 150 g serving per week, the rest of Australian general population up to three 150 g servings per week and Australian children (up to 6 years) one 75 g serving of Australian wild SBT per week (Table 1). The influence of body weight of the consumer should be noted when interpreting exposure assessments. If a body weight of 100 kg is used for the general population, then 772 g of SBT (or five 150 g serves) may be consumed per week. For farmed Australian SBT, the number of servings which can be consumed per week increases due to the lower mercury content of farmed SBT (Yamashita et al, 2005; Padula et al 2008).

The Japanese Ministry of Health, Labour and Welfare issued in 2005 "Advice for Pregnant Women on Fish Consumption and Mercury" (Ministry of Health, Labour and Welfare, 2005b). This Japanese Government advice states that pregnant woman can consume up to two 80 g servings of SBT (not stated if applicable to wild, farmed or both) per week (a total of 160 g) (Ministry of Health, Labour and Welfare, 2005b). This is in good agreement with the dietary exposure results for wild Australian SBT reported in the present study (Table 1). For all other bluefin tuna species, a single 80 g serving may be consumed once per week by Japanese pregnant women, while for the rest of the general Japanese population they are advised to eat a wide

variety of fish and shellfish, to maximise the public health benefits from regular seafood consumption without focusing on eating particular species of fish and or shellfish (Ministry of Health, Labour and Welfare, 2005b).

Japanese research has reported total and methylmercury concentrations in triplicate from multiple cuts (akami, chu-toro and o-toro) of Australian farmed SBT, Japanese wild ABT and Mexican, Maltese and Turkish farmed PBT and ABT (Kawakami et al, 2010). For Australian farmed SBT the mean total mercury concentration (all cuts) was reported as 0.18 mg/kg (range 0.14-0.23) and for methymercury 0.16 mg/kg (range 0.13-0.20); farmed Mexican, Maltese and Turkish farmed PBT and ABT were reported as having a mean total mercury concentration (all cuts) of 0.46 mg/kg (range 0.16-0.67) and for methylmercury a mean concentration of 0.35 mg/kg (range 0.13-0.56); Japanese wild ABT was reported as having a very similar mean total mercury concentration of 0.45 mg/kg (range 0.33-0.61) (all cuts) to wild SBT in the present study and for methylmercury as having a mean concentration of 0.36 mg/kg (range 0.29-0.45) (Kawakami et al, 2010). Regardless of tuna species, the fattier cuts such as the o-toro and the chu-toro had lower total and methylmercury concentrations than the leaner akami cut. This is because protein is composed of sulphur-containing biological thiols such as cysteine (a selenium-containing amino acid), which have a high affinity for mercury, and structural changes in the protein occur when mercury binds to protein-containing tissues; deactivation of the protein may occur from direct toxicity of mercury (Harris et al, 2003). Earlier Japanese research reported that the

majority of methylmercury in wild ABT muscle is stored in myofibrillar and sarcosplasmic proteins (Arema and Umemoto, 1976).

Other Japanese research reported mean total mercury concentrations for Japanese wild caught SBT (n=7) of 0.27 mg/kg and a mean methylmercury concentration of 0.19 mg/kg, giving a mean methylmercury to total mercury percentage of 71% (Yamashita et al, 2005). Fish had a mean body weight of 40.3 kg. Australian farmed SBT (n=7) were also reported in the same study, with a mean total mercury concentration of 0.3 mg/kg and a mean methylmercury concentration of 0.19 mg/kg, giving a mean methylmercury to total mercury percentage of 64%. The body weight of these SBT was 22.5-31.5 kg. As it was not possible to record body weight in the present study, it is not feasible to directly compare wild SBT results; conversion factors do exist for other tuna species but not SBT (Hsu et al, 2000). These total mercury concentration are in good agreement with other studies in which a mean total mercury concentration for Australian farmed SBT was reported of 0.31 mg/kg but should be considered with caution due to the low sample numbers (Padula et al, 2008).

Regulatory standards are set in Japan for mercury in foods, known as provisional regulatory limitations for total mercury and methylmercury in seafood within the Food Sanitation Law, at 0.4 mg/kg for total mercury and 0.3 mg/kg for methylmercury (Japan External Trade Organisation, 2010). However, all tunas are exempt from these provisional regulatory limitations (Japan External Trade Organisation, 2010). The reason for this exemption in the Japanese regulatory

standards is in part due to differences in dietary consumption when the Food Sanitation Law was enacted in 1947, and the availability of reliable analytical methods for the detection of trace levels of mercury in foods at that time. Reliable public health information on the harmful effects of mercury was not widely available until after the Minamata and Iraq incidents occurred and were reported in the international scientific literature (Clarkson et al, 1976; Weiss, 2007). Furthermore, it was not until epidemiological methods had been tested and confirmed by the United States Supreme Court that epidemiological investigation tools such as the Bradford-Hill criteria developed to prove causation became internationally adopted for investigation of environmental contaminants and links to public health outcomes (Bradford-Hill, 1965).

The most recent risk assessment of mercury in seafood by the Japanese Food Safety Commission did not consider bluefin tunas but instead focused on smaller wild tuna species including Yellowfin Tuna (*Thunnus albacores*), Skipjack Tuna (*Katuswonus pelamis*), Dogtooth Tuna (*Gymnosarda unicolour*) and Bonito (*Euthynnus affinis*) (Food Safety Commission, 2004). The reason for this omission can be explained by the tuna species consumed in the Japanese market: SBT 2%, all other bluefin tunas 3%, Yellowfin Tuna 34%, Albacore Tuna (*Thunnus alalunga*) 19% and Bigeye Tuna (*Thunnus obesus*) 35%, while all other tuna species make up the remaining 7% (Sono, 2008; Zhang et al, 2009).

The importance of other everyday food items and their contribution to dietary mercury intake are now being examined in countries such as the Seychelles. Research attention there is now being directed to

seasonal consumption of other food groups such as poultry products which may be reared on diets containing marine fish ingredients (by-products of canning and fish processing) in response to changing dietary sources of mercury (Robinson and Shroff, 2004). Non-seafood food groups have largely been ignored in exposure assessment studies due to the belief they have a negligible contribution. Even if the mercury content of these products is low, intake of poultry products may be quite high; in Australia adult males on average consume in excess of 150 g per day of chicken meat (Food Standards Australia New Zealand, 2008).

It has been speculated, and confirmed for some tuna species, that mercury content is related to the size of the fish and hence public health risk to consumers (Ueda and Takeda, 1977). The present study shows there is a significant relationship between fork length and total mercury content in wild Australian SBT ($p<0.001$). Another factor in the accumulation of mercury in fish is the diet (Suzuki and Hatanaka, 1975; Matsunaga, 1978). Wild SBT consume predominantly a diet of schooling baitfish including Australian sardine (*Sardinops sagax*) (Jenyns, 1842) and red bait (*Emmelichthys nitidus nitidus*) (Richardson, 1845) in addition to locally available crustacean and shellfish species (Young et al, 1997; Froese and Pauly, 2011).

It is important to understand and appreciate the technical basis of mercury standards applied to fish such as tunas in trade. Without access to this basic information and qualifying documentation tuna exporters may be subject to testing and application of standards which

may affect their commercial market position. A summary of a range of international mercury standards applicable to tuna are given in Table 2. However, comparison of international mercury Maximum Levels (MLs) even if numerically identical needs to be done with caution when evaluating published studies due to a number of reasons. These include that different data sets relied upon by individual countries' competent authorities for setting domestic standards may have been generated based on locally available fish items; public health exposure assessments may have been conducted using differing body weights to derive acceptable intake values, domestic dietary habits may differ; definition of the residue (e.g. methylmercury or total mercury) may differ depending on analytical capability; statistical treatment of data sets may differ such as handling of non-detected values; different usage patterns of edible and non-edible portions (inclusion or exclusion of skin and or bones) may apply, hence the same fish may produce different risk profiles depending on portion consumed; measurement methods and ability to detect mercury in different food matrices at relevant concentrations may differ; application of sampling methods may differ for selecting fish for testing to derive occurrence data from imported shipments; composite or individual samples may be used to derive occurrence data and local interpretation of regulatory standards for imported products may vary.

In conclusion, based on the dietary modelling reported in the present study, the Australian general population can consume up to three 150 g serves of wild Australian SBT weekly without exceeding the PTWI. For farmed Australian SBT, a larger serving size may be consumed due to

its lower mercury content. Japanese Government port of entry testing results agree with mercury content results presented in the present study, confirming Australian wild and farmed SBT have the lowest mercury content of the bluefin tunas traded in Japan. A significant association was found between fork length of wild SBT and mercury content; in the present study smaller tunas have less mercury than larger tuna. Mercury content of fish is regulated differently in each country; technical differences between the exporting and importing country's standards, even if these are numerically identical, may mean that fish in international trade may be subject to very different testing and application of importing country's national standards during port of entry inspection which may not be equivalent to those applied at the point of export. In addition, care should be taken when interpreting consumption advisories due to their use of fixed population body weights which may not accurately reflect current actual consumers' body weights and hence public health risk.

Acknowledgements

The Australian Government Australian Fisheries Management Authority (AFMA) is thanked for providing SBT samples. This research was financially supported by a grant from the Australian Government Fisheries Research and Development Corporation (FRDC) and the Aquafin CRC. We would like to thank Dr Andreas Kiermeier of the South Australian Research and Development Institute for reviewing the manuscript and providing helpful comments. We would also like to thank Mr Tim Tanaka (Food South Australian office, Osaka, Japan) for assistance with translation and collation of Japanese documents. Mr

Phil Bridgen of AsureQuality (formerly AgriQuality) is thanked for his technical assistance with mercury analysis.

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CHAPTER SIX

Manuscript in preparation

Padula, D.J.; Daughtry, B.J.; Flint, R.; Nowak, B.F. Mercury content of Australian canned flavoured Skipjack Tuna (*Katsuwonus pelamis*) products. In preparation.

1. Introduction

Mercury is a naturally occurring metallic element of public health interest due to its neurotoxic affects in the developing brain and its ability to bio-accumulate in fish of commercial importance for human consumption (Rasmussen et al, 2005). Worldwide mercury has found many useful applications in our day to day lives in part due to its ability to exist in three different oxidation states (Hg^0, Hg^{1+} and Hg^{2+}) giving it unique chemical properties for use in dental amalgams, thermometers, barometers, sphygmomanometers, preservatives, fungicides, medicine, light switches, smoke detectors, gold mining, neon vapour signs, production of chlorine, and in its crude cinnabar form (HgS), as a paint (Iwai et al, 1977; Bycroft et al, 1982; Van Zyl, 1999; Kosalec et al, 2009; Ryo et al, 2010). Its origin in the marine environment is principally from natural sources such as volcanic eruptions but is also released during combustion of fossil fuels (Nriagu and Becker, 2003; Pyle and Mather, 2003; Itano, 2007). Mercury compounds released from these sources are then transformed from inorganic into more toxic organic forms of mercury such as methylmercury (a neurotoxin) through the action of marine bacteria (Balshaw et al, 2007). The principal route of exposure to methylmercury is from foods of marine origin (Shishido and Suzuku, 1974; Nakagawa, 1995; Nakagawa et al, 1997; Khansari et al, 2005; Zhang et al, 2009). The ability to thermally preserve foods via canning allows seasonally available fish species to be continually available; however this has made possible exposure to mercury, in particular, methylmercury, year round (Berry and Pflug, 2003).

Canned fish products provide a convenient, affordable, shelf stable source of protein and other nutrients of public health importance such as essential fatty acids. However, some fish species used for canning may also contain undesirable levels of mercury (Zhang et al, 1999; Ashraf, 2004; Ikem and Egiebor, 2005; Rasmussen et al, 2005; Mozaffarian and Rimm, 2006; Usydus et al, 2008; Diaz and Hu, 2009; Miklavčič et al, 2011; Mazej and Horvat, 2011). Mercury content of tunas can be broadly grouped according to trophic order, which reflects the dietary sources of each; in general larger species have higher mercury content (Storelli et al, 2010). Worldwide, the main canning species of tuna of commercial importance are Skipjack Tuna (SJT) (*Katsuwonus pelamis*), Yellowfin Tuna (YFT) (*Thunnus albacores*), Albacore Tuna (AT) (*Thunnus alalunga*) and Longtail Tuna (LT) (*Thunnus tonggol*) (Collette and Naven, 1983). SJT is of particular economic importance for canning due to its high yield of meat of 70 % (Balagon and Talabi, 1985). In Australia, available canned tuna products are predominantly produced elsewhere, with Australia importing AUD$221 M in canned tuna products during the 2009/2009 period (Pham, 2010). In the same period Australia exported $1.3 M (Australian Dollars) in canned tuna products to several countries including New Zealand and the United States.

Knowledge on the public health risks of mercury in food products has been largely informed by epidemiological study of two accidental poisoning events: consumption of contaminated fish in Minamata, Japan in the 1950s and of contaminated grain in Basra, Iraq in the 1970s (Clarkson et al, 1976; Hathaway, 1993; Kindaichi and Matsuyama, 2005;

Weiss, 2007; Harada, 2009). In addition, large longitudinal erpidemiological studies have been undertaken in fish eating populations from the Seychelles and Faroe Islands. However in each of these studies different measures of assessment have been used to evaluate mercury exposure, potentially confounding interpretation of results (Davidson et al, 1995; Myers et al, 2000; Rice et al, 2003). Informed by these studies, countries such as Australia regulate total mercury content in canned fish products through statutory limits but also use public consumption advisories targeted to vulnerable population groups such as pregnant women or women considering pregnancy (Abbott et al, 2003; Food Standards Australia New Zealand. 2004a; Food Standards Australia New Zealand, 2004b; Food Standards Australia New Zealand. 2011c; Food Standards Australia New Zealand, 2011d; Food Standards Australia New Zealand, 2011e). For products in international tradem, the Codex Alimentarius Commission provides default methylmercury Guideline Levels which are recognised by the World Trade Organisation for dispute resolution (Hathaway, 1993; Tacon and Metian, 2008). Australia has adopted a consistent approach with Codex in that it is assumed for the purpose of risk assessment that all mercury present in seafood is present as methylmercury (Amano, 1976; Harris, 2003; Honda et al, 2006; Pikholz and Simmons, 2006; Itano, 2007; New Zealand Food Safety Authority, 2008; Satoh, 2008; Pikholz and Simmons, 2010). This assumption allows risk assessment studies to be undertaken based on total mercury measurements in food products rather than methylmercury thus allowing comparison of multiple data sets. This work should be undertaken when food formulations such as

in the present study change to supply new data for risk assessment purposes.

2. Aim of study

The aim of this study was to benchmark total mercury content of mixed varieties of Australian canned SJT products against Australian regulatory standards and public consumption advisories through dietary exposure assessment. This necessitated broader consideration of issues of consumer transparency relating to disclosure of tuna canning species against relevant Australian standards.

3. Materials and methods

3.1 *Sample collection*

SJT samples were provided in original sealed cardboard trays of 12 cans (100 g net weight) direct from the cannery in 2005 and 2006. Condiment ingredient samples were also tested, with each supplied in individual labelled plastic bags from current production lots. These were black pepper powder, butter, cardamom powder, chilli powder, cinnamon powder, clove powder, coriander powder, crushed garlic, cumin powder, curry powder, dried green capsicum, dried red capsicum, dry roasted onion, fennel powder, fenugreek powder, fresh diced onion, minced ginger, salt, season-all (flavoured salt), sugar, tomato flavour, tomato paste and tomato powder. Each ingredient was tested separately.

3.2 *Sample processing*

Each can of tuna was prepared following the procedure from the United States (US) Code of Federal Regulations, Title 21 Food and Drugs, Section 160.190 Canned Tuna (United States Government Printing Office, 2011). In summary this required draining each individual can, with the drained edible contents used for analysis. The drained mass of each can was individually homogenised in a stainless steel HOBART™ food processor. Condiment ingredients were also homogenised individually.

All equipment, tables, benches, knives, food processor and implements were cleaned with DECON90 laboratory detergent and double rinsed with mains tap water before and after each sample was processed. All sample bags were double bagged to protect sample integrity and stored at -80°C until despatch to the laboratories for analysis (no longer than 30 days). Samples were couriered to the laboratories in a sealed foam box in which several freezer gel packs were placed. Laboratories stored samples at -20°C upon arrival. Samples were thawed at 4°C for 24 hours, with all thawing juices included in the defrosted analytical sample. Analysis began one day post arrival of samples at each laboratory.

3.3 Mercury determination

Analysis was limited to total mercury content as methylmercury has been shown to be unstable at freezer temperatures in stored fish samples; significant losses of more than half have been reported following storage from as little as 15 days at -25°C (Devai et al, 2001). In

contrast, total mercury content of biological materials has been shown to be unaffected by freezer storage (De Boer and Smedes, 1997).

Total mercury determination was undertaken in 2005 by an Australian laboratory accredited by the National Association of Testing Authorities (NATA) to the requirements of ISO/IEC 17025 (1999). The method was adapted from United States Environment Protection Agency (USEPA) Method 3050 "Acid Digestion of Sediments, Sludges and Soil", and from preparation stage from USEPA 200.7 Revision 5.0 "Trace Elements in Water, Solids and Bio-solids by Inductively Coupled Plasma-Atomic Emission Spectrometry". A sub-sample (1 g) was digested on a heating block (90°C for 90 minutes) with concentrated nitric acid (Merck, analytical grade) and hydrogen peroxide (Merck, analytical grade) to which stannous chlorine (Merck, analytical grade) in 7% hydrochloric acid (Merck, analytical grade) was added to stabilize the mercury. Sample analysis was performed via Cold Vapor Atomic Absorption Spectroscopy (CETAC M-7500 Mercury Analyser, United States) at an absorbance of 253.7 nm. Blanks and mercury standards (Choice Analytical) 10 μg/L, 100 μg/L, 1000 μg/L were used with each batch of samples (all standards prepared fresh each day). The limit of reporting (LOR) was 0.01 mg/kg (f.w. basis).

Mercury determination in 2006 was undertaken by a New Zealand laboratory accredited by International Accreditation New Zealand (IANZ) to the requirements of ISO/IEC 17025 (1999). An in-house method (Hot Acid Digestion for Food and Raw Materials) was used for the sample preparation. A subsample (0.1 g) was digested using 5 ml of

69% concentrated nitric acid (Aristar, analytical grade) and 0.1ml of 40 % concentrated hydrofluoric acid (Aristar, analytical grade) at 100°C for one hour. The final volume was made up to 50 ml using polished water (18MΩ or better). The digested sample was analysed by Inductively Coupled Plasma Mass Spectrophotometer (ICPMS) (Perkin Elmer, Elan 9000, United States). A blank and three mercury calibration standards (0.1ppb, 1ppb and 10ppb) (Choice Analytical) were run to obtain a correlation coefficient >0.997.

3.3.1 *Quality assurance and quality control*

The contract laboratory in 2005 ran every 10th sample in duplicate and every 20th sample was spiked. The spiked sample recoveries were on average 94% (range 74-120). Duplicates had a relative percent deviation on average of 15% (range 10-32). Blanks were all less than the Limit of Reporting (LOR) of 0.01 mg/kg.

In 2006 the contract laboratory ran acid digest blanks and duplicates every 10 samples. No duplicates showed greater than 10% difference. In house reference material prepared from milk powder (MS05) was analysed after every 20 samples. An ongoing internal laboratory control chart plotted for this reference material recorded mercury results between 95 and 105 ppb. Every 10th sample was spiked, with all recoveries falling between 80 and 120 %. Blanks were all less than the LOR of 0.01 mg/kg.

The limit of reporting (LOR) in both years was 0.01 mg/kg (fresh weight basis). All results were corrected for recoveries in both 2005 and 2006. All results are expressed on a fresh weight basis.

3.4 Dietary exposure assessment

A deterministic exposure assessment to evaluate public health risk of consumption of SJT is reported in Table 1. This was performed following the methodology of Food Standards Australia New Zealand (Food Standards Australia New Zealand, 2004a). It was based on the assumption that Australian women and Australian women planning pregnancy have a body weight of 66 kg, the Australian general adult population a body weight of 67 kg and Australian children (up to six years of age) a body weight of 19 kg. A serving size of 150 g was used for all adults while for children a value of 75 g was used. A Permissible Tolerable Weekly Intake (PTWI) of 1.6 μg/kg per kg body weight per week was used for Australian pregnant women and Australian women planning pregnancy, while for all other groups a PTWI of 3.3 μg/kg per kg body weight per week was used. It was assumed that all mercury was present as methylmercury in calculations, with non-seafood items contributing 0.09 % of methylmercury to the diet for all adults and 0.01 % for children.

Food Standards Australia New Zealand undertakes its risk assessments based on total mercury measurements (based on median values), assuming that all mercury present in seafood samples is present as methylmercury. This approach assumes that people only eat one type of fish, with a serving being 150 g for the adult population and 75 g for

children of 6 years or older (Food Standards Australia New Zealand, 2004b).

3.5 International mercury standards

International mercury standards applicable to canned tuna products were tabulated and expressed as mg/kg (Table 2) (United Nations Environment Program, 2002).

3.6 Comparative canned tuna total mercury data sets

For comparative purposes total mercury content results were taken from New Zealand Food Safety Authority (NZFSA) and United States (US) Food and Drug Administration (FDA) surveys of canned tuna products (Thomson and Lee, 2009; Food and Drug Administration, 2011). The New Zealand data were collected by New Zealand Government agencies to help fill data gaps in NZFSA dietary exposure models. This New Zealand study examined canned SJT (n=40), YFT (n=40) and unknown species (n=40) which were purchased from retail outlets in Wellington in 2008 and 2009. All products were from Thai canneries. A further 37 samples of raw Fijian YFT (un-canned product) caught in 2008-2009 were also included. The Limit of Detection (LOD) for all New Zealand analysis was 0.002 mg/kg. Total mercury results forUS canned tuna products (AT) (n=390), LT (n=7) and unknown variety (n=328) were extracted and analysed for the period 1995-2004 (Figure 1). A LOD of 0.01 mg/kg applied to all US results.

3.7 Statistical treatment of data

An Analysis of Variance (ANOVA) was used to test for differences in mercury content due to the effects of Variety e.g. Lemon and cracked pepper, and year within variety. All analyses were performed in R version 2.11 (R Development Core Team, 2011). A significance level of 5% was used. All data have been expressed on a fresh weight basis to allow comparison with Australian regulatory standards.

All total mercury values reported as traces in the New Zealand canned tuna products were treated as being equal to the LOD (0.002 mg/kg) for the purpose of statistical analyses (Thomson and Lee 2009) (Figure 1).

4. Results

Overall, the mean concentration of total mercury found in Australian canned flavoured SJT products was 0.096 mg/kg (range 0.026-0.22) (Table 1). A significant effect on mercury content was found due to the flavour variety ($p<0.001$) and also year within each variety ($p<0.001$). The Chilli variety had the lowest mercury content, while the Mild Indian Curry variety had the highest.

Of the condiment ingredients, black pepper had a total mercury concentration of 0.012 mg/kg and cinnamon of 0.017 mg/kg (both results fresh weight basis). All other condiment results were less than the LOD of 0.01 mg/kg. Total mercury results from the present study, New Zealand and the United States are presented in Figure 1.

An exposure assessment based on the results of the present study found that pregnant Australian women and Australian women

planning pregnancy could consume seven 150 g servings per week, while the rest of Australian general population could consume up to 14 150 g servings per week and Australian children (up to six years) could consume eight 75 g servings of Australian canned SJT products per week without exceeding the PTWI (Table 2). All of the Australian canned SJT products fully met the Australian regulatory standard for mercury of 0.5 mg/kg set by the Australian competent authority, Food Standards Australia New Zealand. As canned tuna products are traded internationally, regulatory standards applying to mercury in canned tuna products are summarised in Table 3 for comparative purposes.

Table 1. Summary total mercury concentrations in Australian canned flavoured Skipjack Tuna (*Katsuwonus pelamis*) products.

Variety	Year	Mean mercury concentration (mg/kg)	*n*
Chilli	2005	0.11 (0.09-0.15)	12
	2006	0.043 (0.035-0.057)	12
	Mean of years	0.078	N/A
Lemon and cracked pepper	2005	0.11 (0.08-0.13)	12
	2006	0.02 (0.019-0.022)	12
	Mean of years	0.065	N/A
Mild Indian curry	2006	0.17 (0.12-0.22)	24
Onion and tomato savoury sauce	2005	0.09 (0.07-0.11)	12
	2006	0.048 (0.026-0.077)	12
	Mean of years	0.069	N/A
Oven-dried capsicum and chilli	2005	0.10 (0.07-0.12)	12
Oven-dried tomatoes and basil	2005	0.11 (0.08-0.14)	12

Variety	**Year**	**Mean mercury concentration (mg/kg)**	***n***
	2006	0.053 (0.039-0.079)	12
	Mean of years	0.079	N/A
Sweet seeded mustard	2005	0.11 (0.08-0.12)	12
Tomato salsa	2006	0.11 (0.07-0.13)	24
Zesty vinaigrette	2005	0.10 (0.09-0.12)	12
All varieties in this study	N/A	0.096	192

Note: Values in parenthesis represent the range.

Table 2. Dietary exposure assessment for methylmercury from consumption of Australian canned flavoured Skipjack Tuna (*Katsuwonus pelamis*) products.

Exposure assessment inputs	**Australian pregnant women and women planning pregnancy**	**Australian general population**	**Australian children (up to 6 years)**
Permissible Tolerable Weekly Intake[1]	1.6 μg/kg body weight/week	3.3 μg/kg body weight/week	3.3 μg/kg body weight/week
Body weight	66 kg	67 kg	19 kg
Serving size	150 g	150 g	75 g
Total permitted methylmercury intake per week	105.6 μg/week	221.1 μg/week	62.7 μg/week
Estimated methylmercury intake from non-seafood items in the diet (main source spices)	0.94 μg/week (0.09 % of total methylmercury exposure from all foods)	1.14 μg/week (0.09 % of total methylmercury exposure from all foods)	3.10 μg/week (0.01 % of total methylmercury exposure from all foods)
Amount of methylmercury that can be consumed from fish sources	= 104.6-0.94 μg/week = 104.66 μg/week	= 221.1-1.14 μg/week = 219.96 μg/week	= 62.7-3.10 μg/week = 59.60 μg/week
Maximum amount of canned Skipjack Tuna that (SJT) can be consumed per week (assuming	= 104.66 μg/week ÷ 100 μg/kg = 1.05 kg canned SJT tuna/week = 7.0 serves canned SJT tuna/week	= 219.96 μg/week ÷ 100 μg/kg = 2.20 kg canned SJT tuna/week = 14.7 serves canned SJT tuna/week	= 59.60 μg/week ÷ 100 μg/kg = 0.60 kg canned tuna/week = 8.0 serves SJT/week

Exposure assessment inputs	**Australian pregnant women and women planning pregnancy**	**Australian general population**	**Australian children (up to 6 years)**
median total Hg concentration of 100 µg/kg)[1]	7 serves canned SJT tuna/week	14 serves canned SJT tuna /week	8.0 serves canned SJT tuna/week

[1]Assuming that all mercury was present as methylmercury for the purpose of this calculation

Table 3. Summary of international mercury standards which apply to canned tuna products (United Nations Environment Program, 2002).

Country	Portion to which the standard applies	Standard (mg/kg)	Basis of standard	Notes
Australia	Edible content as ordinarily consumed	0.5	Total mercury	Compliant if overall total mercury concentration in 5 sample units is ≤ 0.5 mg/kg
Canada	Not specified	0.5	Total mercury	
Codex Alimentarius Commission	Not specified	0.5	Methylmercury	Guideline value
European Union	Not specified	0.5	Total mercury	
Hong Kong Special Administrative Region of the People's Republic of China	Not specified	0.5	Total mercury	
Japan	N/A	Not set	N/A	All tuna species are exempt
Kingdom of Norway	Not specified	0.5	Total mercury	
Kingdom of Thailand	Not specified	0.5	Total mercury	
Malaysia	Not specified	0.5	Methylmercury	
New Zealand	Edible content as ordinarily consumed	0.5	Total mercury	Compliant if overall total mercury concentration in 5 sample units is ≤ 0.5

Country	**Portion to which the standard applies**	**Standard (mg/kg)**	**Basis of standard**	**Notes**
				mg/kg
People's republic of China	Not specified	0.5	Methylmercury	
Republic of Cuba	Not specified	0.5	Total mercury	
Republic of India	Not specified	0.5	Total mercury	
Republic of Korea	Not specified	0.5	Total mercury	
Republic of Mauritius	Not specified	1	Total mercury	Action level.
Republic of Senegal	Not specified	0.5	Total mercury	Threshold limit
Republic of Singapore	Not specified	0.5	Total mercury	
Republic of Zimbabwe	Not specified	0.5	Methylmercury	
Socialist Republic of Vietnam	Not specified	0.5	Total mercury	
Taiwan	Edible portion	0.5	Methylmercury	
United States of America	Not specified	1	Methylmercury	

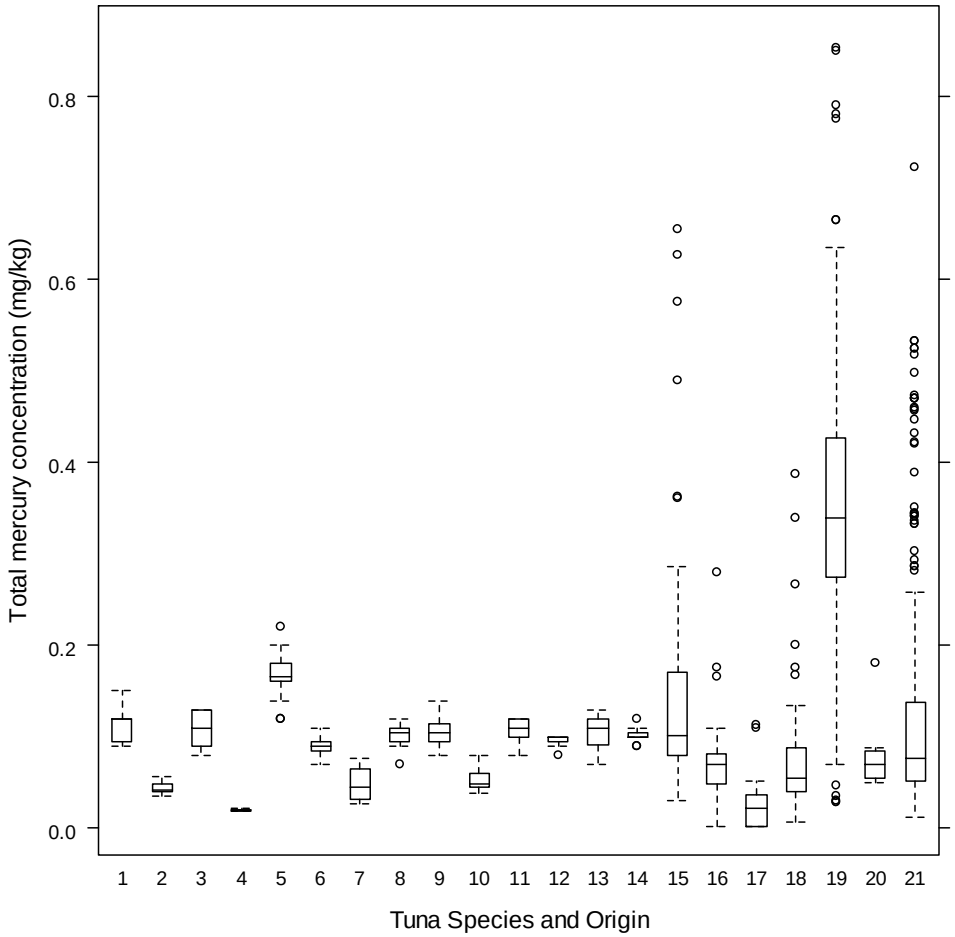

Figure 1. Total mercury content of Australian canned Skipjack Tuna (*Katsuwonus pelamis*) (the present study – hereafter referred to as PS) and mixed tuna species canned products of New Zealand and United States retail origin. Tuna species and origin: 1. Chilli 2005 (PS) 2. Chilli 2006 (PS) 3. Lemon and cracked pepper 2005 (PS) 4. Lemon and cracked pepper 2006 (PS) 5. Mild Indian curry 2006 (PS) 6. Onion and tomato savoury sauce 2005 (PS) 7. Onion and tomato savoury sauce 2006 (PS) 8. Oven-dried capsicum and chilli 2005 (PS) 9. Oven-dried tomatoes and basil 2005 (PS) 10. Oven-dried tomatoes and basil 2006 (PS) 11. Sweet seeded mustard 2005 (PS) 12. Tomato salsa 2005 (PS)

continued

13. Tomato salsa 2006 (PS) 14. Zesty vinaigrette 2005 (PS) 15. Fresh Yellowfin Tuna (*Thunnus albacares*) (New Zealand [NZ] origin) 16. Canned Skipjack Tuna (NZ origin) 17. Canned Yellowfin Tuna (NZ origin) 18. Canned tuna (NZ origin and of unknown species) 19. Canned Albacore Tuna (*Thunnus alalunga*) (United States [US] origin) 20. Longtail Tuna (*Thunnus tonggol*) (US origin) 21 Canned tuna (US origin and of unknown species).

5. Discussion

Total mercury content of all SKJ products tested was low when judged against Australian regulatory standards. However, variability exists in the total mercury content found in the present study between varieties and years. Differences in total mercury content between years in the same variety may be due to origin of SJT, size of tuna and seasonal differences in fish such as fat content. It has been reported that in farmed Southern Bluefin Tuna (*Thunnus maccoyii*) mercury content varies seasonally in response to lipid content fluctuations caused by water temperatures changes and dietary inputs (Balshaw et al, 2008a; Balshaw et al 2008b). This inherent variability in living animals such as fish, means that well structured experimental design is particularly important when generating mercury data for risk assessment purposes (Voegborlo et al, 1999; Storelli et al, 2010).

The results of the present study are contrasted with those reported in a Fijian study undertaken to generate mercury occurrence data for informing a risk assessment of locally available fish species which

examined Fijian canned SJT (n=9), AT (n=6) and unknown tuna species (n=3) (Kumar et al, 2006). The study found a mean total mercury concentration in SJT of 0.08 mg/kg (range 0.06-0.11), in AT of 0.20 mg/kg (range 0.16-0.27) and in unknown tuna species of 0.09 mg/kg (0.05-0.16). It is speculated that the reason for the lower total mercury content in the SJT products in this study compared with the present study could be due to smaller tuna being used for canning. In addition there would also be a dilution effect due to the addition of condiment ingredients in the present study, making comparison difficult.

Direct statistical comparison of the present study's results with those of other studies' is not valid due to a number of factors, such as unknown can filling liquid (water, oil, brine), potential differences in sample processing between studies (drained versus undrained product), differences in laboratory LODs, different laboratory methods including correction of results for recoveries and treatment of non-detected results, different canning processes used, differences in fish preparation practices in individual canneries, potential for metal transfer from can to tuna in acidic packed media and the potential inclusion of canned products containing tuna bone and or skin and non-disclosed tuna species in many canned products. The canning process itself has been reported as elevating mercury content of AT canned product; separate effects were observed with water and oil canned variants (Rasmussen and Morrissey, 2007). However, on the issue of drained versus undrained product, it has been reported that there is no significant effect on total mercury results in drained versus undrained canned tuna and mackerel products (Burger and Gochfeld, 2004). Consumer

preparation practices in the home may also differ in ways not accounted for in the present study, such as cooking (mercury can exist as a gas) of can contents or consuming other foods at the same meal which may affect absorption of contaminants such as mercury.

Consumers in Australia and elsewhere cannot always readily identify specific canned tuna species due to non-disclosure of canning tuna species on product labels (Caswell, 2006). Once canned it is difficult to discriminate between different tuna species due to the thermal treatment (Mendez and Rehbein, 1999). An Australian fish names system already exists which could be extended to include canned tuna products; this would allow consumers with an interest in the mercury content of these products to make more informed selections (Seafood Services Australia Limited, 2007).

Mercury may also enter the human diet from non-seafood food items of irregular or inconsistent consumption across the population, reflecting indivudal food preparation practices. Spices such as cinnamon and pepper may be contaminated with mercury due to a number of factors related to agricultural production methods, storage and transportation. In humid spice producing countries, stored bulk spices may be fumigated with mercury-containing fungicides to control spoilage fungi and prevent deterioration. Products such as 2-methoxyethyl mercury chloride and phenyl mercury acetate are mercury containing fungicides used in spice storage (Dikshit et al; 1983). There is no Australian standard for mercury in spices. Black pepper (*Piper nigrum*) is harvested from fruit of a tropical flowering vine; worldwide

cultivation is concentrated in India (Bhat et al, 2006). Cinnamon (*Cinnamomum verum*) is harvested from the bark of coppiced trees; cultivation is concentrated in Sri Lanka, with other related cinnamon species cultivated in nearby countries (Barceloux, 2008). The origin of the black pepper and the cinnamon tested in this study is unknown. Given the low dietary intake of these spices, plus the fact that mercury in these spices would have most likely been present in an inorganic form, therefore public health risk is likely to be extremely low.

Worldwide, public consumption advisories issued by food control authorities may inform consumers of the risks of mercury in fish species, but they may be interpreted very differently than originally intended, with some consumers simply avoiding all seafood products when exposed to such messages (Rasmussen et al, 2005; Hughner et al, 2008; Shimshack and Ward, 2010). Public health regulators need to balance the need for public information on potential hazards present in food products against the unintended potential harm from substitution of other foods of lower nutritional value by consumers misinterpreting such advice (Cohen et al, 2005; Rasmussen et al, 2005). Official government advisories for vulnerable population groups such as pregnant women issued by the Japanese Ministry of Health, Labour and Welfare say pregnant women can consume canned tuna products without restriction (Ministry of Health, Labour and Welfare, 2005). In contrast, Food Standards Australia New Zealand allow all Australians (including pregnant women and women planning pregnancy) to consume one can (95 g) of tuna daily (Food Standards Australia New Zealand, 2011c). Both advisories use different language informed by

local food consumption habits to describe the safety of canned tuna products. These consumption advisories are underpinned by many assumptions including the use of fixed body weight values for the Australian population which are inconsistent with current body weight values (Table 2). The most recent Australian body weight data (self reported) reports a mean body weight of 84 kg for adult men (all ages) and a mean weight of 68 kg for adult women (all ages) (Australian Bureau of Statistics, 2008). Hence, the use of fixed body weights in risk assessments instead of actual body weight values may overstate the potential public health risk of mercury in some consumers.

Risk analysis principles implemented through the risk assessment process form the underlying technical basis of most countries' food regulatory institutions (Hathaway, 1993; Sekizawa, 2003; Toyofuku, 2006; Uneyama, 2010). International bodies such as Codex recognise methylmercury as the chemical form of mercury for risk assessment purposes and derivation of threshold values (Pilgrim et al, 2000; Harris et al, 2003; Joint FAO/WHO Expert Committee on Food Additives, 2003; Rice et al, 2003; Satoh, 2003; Codex Committee on Food Additives and Contaminants, 2005; Joint FAO/WHO Expert Committee on Food Additives, 2006; Yamamoto, 2010). However, it should be noted that biological variability in individual human toxicokinetics may not be reflected accurately in single numerical reference intake values; co-exposure to other environmental contaminants such as polychlorinated biphenyls (PCBs) may potentially act synergistically with mercury (Costa, 1988; Dourson et al, 2001).

Internationally, food control authorities apply different regulatory limits for acceptable levels of mercury (Table 3). The technical basis of these regulatory standards is not always apparent even if they are numerically equivalent. Another form of regulatory control that Australia and Papua New Guinea have formerly used to protect consumers from mercury exposure has been through setting catch standards specifying maximum legal size prohibiting the taking of fish including tuna species over specified fork length. This is because fork length is a predictor of total mercury content in some species of Australian and Papua New Guinean fish (Hancock, 1990). Although these standards no longer apply, it is customary practice on boats catching tunas to direct all small tunas for processing for canning as large tuna fetch higher prices when sold whole (pers. comm. Dr John Reeve, New Zealand Food Safety Authority).

In conclusion, the present study provides new total mercury content data for informing public evaluation of Australian canned flavoured SJT products. These products, regardless of flavour variety, had very low levels of total mercury that fully met Australian regulatory standards. This study demonstrated that the general Australian population may safely consume up to 14 150 g serves of these Australian canned SJT products per week without exceeding the PTWI. Further research is needed to investigate origin and mercury chemical form present in condiment ingredients. To provide greater transparency to Australian consumers interested in selecting low mercury content canned tuna products, a nationally recognised scheme such as the Australian Fish Names Standard could be extended to

include canned tuna products, which would allow consumers to identify specific canned tuna species (Seafood Services Australia Limited, 2007). This study also provides a framework which could be used to investigate the mercury content of other Australian fish canning species such as Atlantic Salmon (*Salmo salar*), Australian Sardine (*Sardinops sagax*) and Blue Mackerel (*Scomber australasicus*) to inform public health risk assessments.

Acknowledgements

This research was financially supported by funding from Port Lincoln Tuna Processors Pty Ltd. We would like to thank Dr Andreas Kiermeier of the South Australian Research and Development Institute for reviewing the manuscript and providing helpful comments. We would also like to thank Dr Ian Eckhard of Advanced Analytical and Mr Phil Bridgen of AsureQuality (formerly AgriQuality) for technical assistance with mercury analysis.

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CHAPTER SEVEN

Manuscript in preparation

Padula, D.J.; Nowak, B.F. Causes of detentions and rejections of Australian seafood in international trade. In preparation.

Introduction

Globally in 2008 approximately 37% (based on live weight) of all seafood products were exported with a value of $US102 Billion; this has risen 83% since the year 2000 (Food and Agriculture Organization, 2010). In value terms developed countries accounted for approximately 80% of all imports. Globally, annual consumption of seafood products is estimated at 17 kg per person; with fish accounting for approximately 16% of animal protein and 6% of total protein consumed (Food and Agriculture Organisation, 2010). Seafood products in international trade are subject to importing countries' food safety regulatory standards. Public confidence and perception of imported seafood products may be eroded following media reports of violations of food safety standards (Winter-Nelson, 2009). Some public perceptions of food safety issues in food products may be country or production method specific (Wang et al, 2008; Wilcock et al, 2004).

Seafood may be broken down into two broad production classifications, aquaculture (farmed) or wild capture. Public perception on the hazards potentially contained in products from each of these two broad groups may influence purchasing decisions, whether factually correct or incorrect (Smith and Riethmuller, 1999; Liao, 2009; Santerre, 2010; Rasco, 2010). Some consumers may believe that as veterinary medicines may be legally administered to farmed fish to control diseases and manage aquatic animal health issues that such fish are less safe (Redshaw, 1995). In Australia aquaculture products exported to the European Union (EU) are subject to comprehensive annual testing which includes veterinary medicines from every single producing farm

(The Council of the European Union, 1996; Feazey, 2011). Attestations are then made by the national competent authority of the exporting country to the importing country of the safety of exported product.

Developed countries generally have established food regulatory control systems; developing countries may have limited technical resources and instead rely on standards set by international bodies such as the Codex Alimentarius Commission (McMillan, 1991; Hathaway, 1993; Antle, 1999; Dey et al, 2005; Aginam, 2007). Codex standards are relied upon by the World Trade Organisation (WTO) when settling disputes between member nations (Hathaway, 1999; Athukorala and Jayasuriya, 2003).

Food control institutions may be country or regionally based such as within the EU (Bergeaud-Blacker and Ferretti, 2006). The structure of these institutions is not universal, often responsibilities for different regulatory functions are held by multiple agencies with powers to manage on farm and off farm activities (Tam and Young, 2005; Ni and Zeng, 2009; Ming, 2006; Broughton and Walker, 2010). Australia and New Zealand have a bi-nationally managed food regulatory institution model, with enforcement powers delegated to State and Territory Governments (Gruber et al, 2003; Healy et al, 2003).

The emergence of private purchase standards over and above any national or international market access requirements but with de facto powers has occurred in some markets in response to commercial competitive pressures (Northern, 2001; Henson and Reardon, 2005).

Complicating this further has been the introduction of free trade agreements between countries which may see additional regulatory controls and testing requirements being placed on seafood products in trade between member countries (Omagari, 1997).

Technical capacity to measure food safety hazards (and interpret and assess their risk) in imported seafood products also varies greatly between countries (Chen, 2004; Chen, 2008). Tests applied by the country of origin may not be equivalent to those applied at the port of entry by the competent authority to assess compliance with domestic standards (Lupien and Kenny, 1998; Berg and Licht, 2002). Even if numerically equivalent, standards may be based on differing technical underpinnings. Food safety standards may be viewed as technical non-tariff barriers to trade by some countries due to a lack of transparency and discriminatory application of standards (Thilmany and Barrett, 1997; Huggett et al, 1998; Henson and Caswell, 1999; Hooker, 1999; Lugard and Smart, 2006; Anders and Caswell, 2009; Baylis et al, 2009).

Transparent information is required when interpreting standards such as knowing which tissue is analysed and by which laboratory method (e.g. screening method vs. confirmatory method). The analytical sample may include or exclude skin and or bones; this may alter the reported results and hence its public health significance to the consuming population. The distribution of veterinary medicines in fish has been studied in Japanese farmed Yellowtail (*Seriola quinqueradiata*); oxytetracycline residues were found in order of decreasing concentration in skin, liver and muscle (Abe and Fuchino, 2001). The

inclusion of skin in the analytical sample may elevate reported concentrations of antibiotic substances in farmed fish. For the purpose of trade reporting there are established protocols to be followed for some markets such as the EU (The Council of the European Union, 1996). The EU protocols require for some veterinary medicines that skin is included in the analytical sample and others that skin is excluded (The Council of the European Union, 1996; European Commission, 2004).

Microbiological requirements have been introduced by some countries in response to domestic food safety concerns. Examples include Singapore's requirements for pre-export testing and certification of oysters for norovirus (Liat, 2007; Stenhouse, 2008). Other countries such as China are introducing new pathogen limits for imported seafood products including for *Salmonella, Listeria monocytogenes* and *Vibrio parahaemolyticus* (Ministry of Health, 2010).

The aim of this study was to identify all publicly available food safety related causes of rejections and detentions of Australian seafood in international trade over the period 2000-2011 and compare port of entry regulatory standards applied in each of these cases to those applied in Australia.

Methods

Public reports of detentions and rejections were searched for on the internet using the internet search engine Google and competent authority websites of importing countries for Australian seafood entries

(General Administration of Quality Supervision Inspection, Inspection and Quarantine, 2011a; General Administration of Quality Supervision Inspection, Inspection and Quarantine, 2011b; European Commission, 2011; Food and Drug Administration, 2011; Ministry of Health, Labour and Welfare, 2011; Food and Environmental Hygiene Department, 2011; Food Inspection Agency, 2011). Information was accessible for Canada, China, EU, Hong Kong, Japan, New Zealand and the United States. Not all countries publish publicly accessible notification reports or they are not avilable in English. Chinese language notification reports were translated into English by Mr Simon Liu (Seafood Services Australia). All notification reports were tabulated and summarised by country, year and product (Table 3). Notifications retrieved for the years 2000 to 2011 (not all countries provided information over this period) were searched. Measurement units were standardised from original notification reports. Significant figures in original reports were not corrected but are reported as per original notifications. Notifications were grouped according to causes (Table 1). Notifications presented in the current study (by country) cover 87% of Australian exported seafood by value (2008/2009 period) and 76% by tonnage for the same period and their contribution to total notifications was calculated as a percentage (Table 1). The remaining countries do not provide publicly accessible notifications of detentions or rejections of seafood products. Some countries such as the EU provide a searchable database of all Rapid Alert System for Food and Feed (RASFF) notifications back to 1979 (European Commission, 2011).

Results

Overall there were 63 publicly available detentions or rejections reports of Australian seafood products over the period 2000 to 2011 (Table 3). Of these, 70 % of the total notifications were for cadmium, 6 % were for sulphur dioxide, 5 % were for histamine, 3 % were for polyphosphates and all other causes accounted for 2 % each (ciguatera toxin, crystal violet, *E. coli*, excessive bacteria, lead, parasites, poor hygiene, poor temperature control and *Vibrio parahaemolyticus*) (Table 3).

Australia imports 200,000 T of seafood per annum (Moir, 2009). In the 2007/2008 period $1.13 Billion (Australian Dollar) of seafood was imported into Australia (Moir, 2009). In Australia, seafood products ranked as low risk are sampled at a rate of 1 in 20 shipments against specified tests, while risk categorised seafood products are sampled at a rate of up to 100% (Department of Agriculture, Fisheries and Forestry, 2008; Department of Agriculture, Fisheries and Forestry, 2009). Other developed countries adopt similar risk-based treatment for sampling and testing of imported seafood products (Valdimarsson, 2004; Ababouch et al, 2005).

In the 2009/2010 period Australia exported 46,900 T of seafood with a total value of $1.1 Billion (Australian Dollar) (Pham, 2010). Hong Kong is the leading (by value) importer of Australian seafood (Pham, 2010; Table 1). Product may be further processed in China and re-exported elsewhere. The EU imports Australian seafood products into six member nations (Table 2). Australian fisheries statistics do not provide a breakdown of all individual markets or if products are of aquaculture

or wild capture origin. These undifferentiated market results have been pooled and are reported as “Other” in Table 2.

Table 1. Australian seafood exports markets summary - all countries for 2009/2010 period including public import notifications (Pham, 2010).

Rank	Country	Public import notifications	Tonnage	Value $AD
1	Hong Kong	0	12,113	525,286,000
2	Japan	8	15,599	302,258,000
3	United States	1	1,927	64,403,000
4	Chinese Taipei	Not available	2,989	53,744,000
5	Singapore	Not available	1,815	43,713,000
6	China	4	2,575	29,796,000
7	European Union	50	1,390	28,353,000
9	Malaysia	Not available	784	12,545,000
10	New Zealand	0	2,176	9,154,000
11	Thailand	Not available	1,506	7,328,000
12	Indonesia	Not available	730	4,557,000
13	Vietnam	Not available	451	3,249,000
14	Canada	0	97	2,860,000
8	Other	Not available	2,748	18,168,000

Rank	Country	Public import notifications	Tonnage	Value $AD
TOTALS		63	46,900	1,105,414,000

Note: Excludes live seafood (approximate value $50M, unknown tonnage) and non-edible seafood (e.g. fish meal and pearls)

European Union countries include: France, Greece, Portugal, Spain and the United Kingdom

Table 2. Australian seafood exports to European Union member nations for 2009/2010 period and public import notifications (Pham, 2010).

European Union member nation	Public import notifications	Tonnage	Value AUD$
Portugal	4	18	218,000
Greece	6	45	714,000
United Kingdom	3	79	1,489,000
Italy	6	184	2,229,000
Spain	31	545	4,207,000
France	0	519	19,496,000
TOTALS	50	1,390	28,353,000

Table 3. Summary list of international notification reports by notifying country of Australian seafood products for the period 2000-2011 (General Administration of Quality Supervision Inspection, Inspection and Quarantine, 2011a; General Administration of Quality Supervision Inspection, Inspection and Quarantine, 2011b; European Commission, 2011; Food and Drug Administration, 2011; Ministry of Health, Labour and Welfare, 2011, Food and Environmental Hygiene Department, 2011, Food Inspection Agency, 2011). All units mg/kg unless otherwise given.

No.	Country	Date	Original reported species information	Cause	Reported results	Importing country standard	Australian standard	Fate of shipment
1	China	30/5/2007	Prawns and squid	Cadmium and lead	N/A	0.5	Not set	Not known
2	China	29/8/2007	Crabs	Cadmium	N/A	0.5	Not set	Not known
3	China	20/12/2008	Oysters	Cadmium	0.32	0.5	Not set	Not known
4	China	4/9/2009	Rock Lobster	*Vibrio parahaemolyticus*	Detected	Nil	Not set	Not known
5	China	20/5/2010	Yellowtail Kingfish (*Seriola lalandi*)	Crystal violet	0.84 μg/kg	0	Not set	Shipment destroyed.

No.	Country	Date	Original reported species information	Cause	Reported results	Importing country standard	Australian standard	Fate of shipment
6	Greece	30/3/2006	King Prawns (*Melicortus latisulcatus* and *Melicortus plebejus*)	Cadmium	0.933	0.5	Not set	Not known
7	Greece	8/5/2006	Prawns (*Metapenaeus endeavouri* and *Metapenaeus ensis*)	Cadmium	0.677	0.5	Not set	Re-despatched.
8	Greece	1/8/2006	Frozen King Prawns	E452 polyphosphates	N/A	5	Not set	Notification withdrawn and shipment released.
9	Greece	1/8/2006	Frozen King Prawns	E452 polyphosphates	N/A	5	Not set	Notification withdrawn and shipment released.

No.	Country	Date	Original reported species information	Cause	Reported results	Importing country standard	Australian standard	Fate of shipment
10	Greece	17/7/2007	Frozen prawns	Cadmium	0.76	0.5	Not set	Re-despatched.
11	Greece	12/12/2007	Frozen prawns	Cadmium	0.906	0.5	Not set	Re-despatched.
12	Italy	4/8/2003	Pargo (Snapper)	Parasites	N/A	0.5	Not set	Re-despatched.
13	Italy	20/9/2005	Frozen whole prawns (*Metapenaeus endeavouri* and *Metapenaeus ensi*)	Cadmium	0.7	0.5	Not set	Destroyed.
14	Italy	12/12/2005	Frozen black tiger shrimps (*Penaeus monodon*)	Cadmium	0.735, 0.789	0.5	Not set	Re-despatched.
15	Italy	12/12/2005	Frozen black tiger shrimps (*Penaeus monodon*)	Cadmium	0.666, 0.646, 0.750, 0.621, 0.880, 0.853, 0.883	0.5	Not set	Re-despatched.
16	Italy	30/11/2009	Fresh Yellowtail Kingfish (*Seriola lalandi*)	Histamine	1,000	200	200	Product already consumed.

No.	Country	Date	Original reported species information	Cause	Reported results	Importing country standard	Australian standard	Fate of shipment
17	Italy	3/2/2010	Yellowtail Kingfish (*Seriola lalandi*)	Histamine	135, 149, 99, 180, 101, 102, 154, 180	200	200	Distribution on the market allowed.
18	Japan	1/2/2007	Frozen lobster American sauce	Undesignated additive (polysorbates)	N/A	5,000	1,000	Detained at Kobe port.
19	Japan	1/4/2007	Canned abalone	Sulfur dioxide	66	30	1,000	Detained at Osaka port.
20	Japan	1/6/2007	Chilled squaretail coral grouper (*Plectropomus areolatus*)	Poisonous fish (ciguateric fish)	N/A	Not set	Not set	Detained at Narita airport.
21	Japan	1/6/2009	Frozen tiger shrimps	Sulphur dioxide	23	100	30 (raw), 100 (cooked)	Not known.

No.	Country	Date	Original reported species information	Cause	Reported results	Importing country standard	Australian standard	Fate of shipment
22	Japan	1/11/2009	Live oysters	Violation of compositional standard *E. coli*	MPN 330/100 g	230/100 g	7/g	Detained at Narita airport.
23	Japan	1/12/2009	Canned abalone	Disodium Pyrosulfite (as sulphur dioxide)	36	30	1,000	Detained at Tokyo port.
24	Japan	1/10/2010	Frozen sun dried mullet roe	Live bacteria count	7.4×10^5/g	Not set	Not set.	Detained at Narita airport.
25	Japan	2/10/2010	Canned abalone	Disodium Pyrosulfite (as sulphur dioxide)	41	30	1,000	Detained at Nagoya port.
26	Portugal	21/4/2006	Banana prawns (*Fenneropenaeus spp*)	Cadmium	0.67, 1.47, 0.66	0.5	Not set	Re-despatched.

No.	Country	Date	Original reported species information	Cause	Reported results	Importing country standard	Australian standard	Fate of shipment
27	Portugal	1/9/2006	Frozen tiger prawns (*Marsupenaeus japonicos*, *Penaeus esculentus*, *Penaeus semisulcatus*)	Cadmium	0.58, 0.59	0.5	Not set	Distribution on the market allowed.
28	Portugal	7/12/2006	Frozen tiger prawns (*Marsupenaeus japonicus*, *Penaeus esculentus* and *Penaeus semisulcatus*)	Cadmium	0.994, 0.579	0.5	Not set	Distribution on the market allowed.
29	Portugal	31/1/2007	Frozen King prawns	Cadmium	0.653	0.5	Not set	Distribution on the market allowed.
30	Spain	26/11/2004	Frozen prawns	Cadmium	0.9	0.5	Not set	Re-despatch
31	Spain	30/11/2004	Prawns (*Metapenaeus endeavouri*)	Cadmium	0.9	0.5	Not set	Re-dispatched.

No.	Country	Date	Original reported species information	Cause	Reported results	Importing country standard	Australian standard	Fate of shipment
32	Spain	30/11/2004	Prawns (*Metapenaeus endeavouri*)	Cadmium	0.56	0.5	Not set	Re-dispatched.
33	Spain	30/11/2004	Prawns (*Metapenaeus endeavouri*)	Cadmium	2.5	0.5	Not set	Re-dispatched.
34	Spain	28/6/2005	Frozen Banana prawns	Cadmium	0.8	0.5	Not set	Re-despatched.
35	Spain	5/7/2005	Frozen raw whole banana prawns (*Fenneropenaeus merguiensis*)	Cadmium	0.8	0.5	Not set	Re-despatched.
36	Spain	5/7/2005	Frozen raw whole banana prawns (*Fenneropenaeus merguiensis*)	Cadmium	0.6	0.5	Not set	Re-despatched.
37	Spain	6/7/2005	Prawns (*Fenneropenaeus spp*)	Cadmium	1.2	0.5	Not set	Re-despatched.

No.	Country	Date	Original reported species information	Cause	Reported results	Importing country standard	Australian standard	Fate of shipment
38	Spain	6/7/2005	Prawns (*Fenneropenaeus spp*)	Cadmium	2.5	0.5	Not set	Re-despatched.
39	Spain	6/7/2005	Prawns (*Fenneropenaeus spp*)	Cadmium	1.3	0.5	Not set	Re-despatched.
40	Spain	6/7/2005	Prawns (*Fenneropenaeus spp*)	Cadmium	1.2	0.5	Not set	Re-despatched.
41	Spain	6/7/2005	Prawns (*Fenneropenaeus spp*)	Cadmium	1	0.5	Not set	Re-despatched.
42	Spain	6/7/2005	Prawns (*Fenneropenaeus spp*)	Cadmium	0.9	0.5	Not set	Re-despatched.
43	Spain	7/7/2005	Frozen raw whole banana prawns (*Fenneropenaeus merguiensis*)	Cadmium	1.0	0.5	Not set	Re-despatched.

No.	Country	Date	Original reported species information	Cause	Reported results	Importing country standard	Australian standard	Fate of shipment
44	Spain	7/7/2005	Frozen raw whole banana prawns (*Fenneropenaeus merguiensis*)	Cadmium	1.6	0.5	Not set	Re-despatched.
45	Spain	7/7/2005	Prawns (*Fenneropenaeus spp*)	Cadmium	0.7	0.5	Not set	Re-despatched.
46	Spain	15/7/2005	Frozen prawns	Cadmium	0.9, 1.3, >2.5, 1.1, 1.9, >2.5, 1.6	0.5	Not set	Re-despatched.
47	Spain	20/9/2005	Frozen whole prawns (*Metapenaeus endeavouri* and *Metapenaeus ensis*)	Cadmium	0.7	0.5	Not set	Re-despatched.
48	Spain	25/11/2005	Whole raw frozen banana prawns	Cadmium	0.6	0.5	Not set	Re-despatched.

No.	Country	Date	Original reported species information	Cause	Reported results	Importing country standard	Australian standard	Fate of shipment
49	Spain	9/1/2006	Frozen whole raw banana prawns (*Fenneropenaeus merguiensis*)	Cadmium	3.24, 1.14, 2.52, 4.18, 4.45, 2.44, 2.54, 4.3, 2.36, 3.08	0.5	Not set	Re-despatched.
50	Spain	2/3/2006	Frozen raw banana prawns	Cadmium	0.55	0.5	Not set	Re-despatched.
51	Spain	30/8/2006	(*Melicertus latisulcatus* and *Metapenaeus endeavouri*)	Cadmium	2.11, 1.43, 1.82	0.5	Not set	Returned.
52	Spain	6/11/2006	Frozen whole raw prawns (*Penaeus spp*)	Cadmium	0.70	0.5	Not set	Re-despatched.
53	Spain	6/11/2006	Frozen whole raw prawns (*Penaeus spp*).	Cadmium	0.86	0.5	Not set	Re-despatched.
54	Spain	18/12/2006	Shrimps (*Penaeus spp*)	Cadmium	1.8	0.5	Not set	Re-despatched.
55	Spain	18/12/2006	Shrimps (*Penaeus spp*)	Cadmium	1.2	0.5	Not set	Re-despatched.

No.	Country	Date	Original reported species information	Cause	Reported results	Importing country standard	Australian standard	Fate of shipment
56	Spain	4/6/2007	Frozen king prawns	Cadmium	0.71	0.5	Not set	Re-despatched.
57	Spain	28/9/2007	Frozen whole prawns	Cadmium	1.12	0.5	Not set	Re-despatched.
58	Spain	10/10/2007	Frozen raw whole Endeavour prawns	Cadmium	1.43	0.5	Not set	Re-despatched.
59	Spain	17/10/2007	Frozen prawns (*Metapenaeus endeavouri*)	Cadmium	0.9	0.5	Not set	Re-despatched.
60	Spain	13/12/2007	Frozen raw prawns	Cadmium	1	0.5	Not set	Re-despatched.
61	United Kingdom	20/9/2010	Yellowtail Kingfish (*Seriola lalandi*)	Poor hygienic state	N/A	N/A	N/A	Chilled product. Destroyed.
62	United Kingdom	5/1/2011	Yellowtail Kingfish (*Seriola lalandi*)	Poor temperature control	N/A	N/A	N/A	Chilled product. Destroyed.
63	United States	2006/2007	Swordfish	Histamine	N/A	50/500	200	Fish from QLD

Note: N/A Information not available

Discussion

Cadmium is a natural contaminant of the marine environment. Previous Australian studies have reported elevated concentrations of cadmium in several species of commercially harvested crustaceans (Darmono and Denton, 1990). Cadmium is not uniformly distributed throughout the prawn body, with the hepatopancreas being the main site of cadmium storage in Australian wild capture prawn species; this means there may be large variation in cadmium results if different portions are tested (information is not available on the distribution and levels of cadmium in Australian farmed prawn species) (Peerzada et al, 1992). Pre-export testing may be undertaken on raw or on cooked product, peeled or unpeeled, head and tail on or off and after deveining (removal of the hepatopancreas). Upon arrival it may be simply tested as a whole prawn. Also, samples may be pooled or tested individually for compliance testing (an analytical sample may require more tissue than can be supplied from one individual prawn). Sample collection, processing, homogeneity and storage conditions do have effects on recovery of some residues and contaminants including pesticides and speciated metallic elements in biological matrices (Lichton, 1992; Thompson and Ramsey, 1995; De Boer and Smedes, 1997; Devai et al, 2001; Krejčová, 2008). Official standards exist for sampling and processing of samples for port of entry testing of imported foods (European Committee for Standardisation, 2002).

Australia has no regulatory standard for cadmium in crustaceans as this seafood category does not represent a high risk to public health (Abbott et al, 2003; Food Standards Australia New Zealand, 2011).

Cadmium has been identified as a naturally occurring contaminant with a problematic occurrence in some seafood products internationally (Figueroa, 2007). In the case of chemicals (does not apply to cadmium) with no specific standard set these chemicals may be regulated through the use of a default standard (sometimes called a uniform limit) applied at 0.01 mg/kg or addressed on a case by case basis in some countries such as Japan (Kawasaki, 2005). However, some chemicals such as melamine and cyanuric acid should not be treated in this manner, particularly when their inclusion in food is not customary or has never been evaluated for food safety implications (Pei et al, 2011).

Port of entry inspection of imported seafood products may be performed on a targeted or random basis by national authorities. In Japan a mixed approach is taken with pre-announced general surveillance of all countries' products, supplemented with country-specific targeted surveillance (Ministry of Health, Labour and Welfare, 2011). In the EU seafood inspection frequency and selection of tests is at the discretion of the national food control authority in each EU member nation (European Commission, 2004). Hence, if a shipment is split and distributed to several EU member nations it may be reported on multiple occasions, but all of the notifications pertain to a single shipment. This may lead to over reporting of violations of importing countries standards which are not reflective of the actual risk. In Table 3 it can be seen that some EU member nations report single numerical cadmium results, while others report multiple and vice versa. The full technical details of each of these notifications is not known fully such as the sampling of the shipment, sample processing, sample storage,

analytical methodology and reporting conventions; caution should be applied when interpreting such tables of numerical violations without access to this qualifying information.

Not all countries have easily accessible transparent food regulatory standards that allow unambiguous comparison of exporting country's standards with those of the importing country. Without this information available to exporters, it may lead to incorrect application or interpretation of regulatory standards. Internationally there is a need for a consolidated online accessible repository for all countries' food regulatory standards; such a system would need to work with non-English language materials but would better assist all seafood exporters to understand individual market access requirements; models for this service already exist for aquatic health (Office International des Epizooties, 2011). This would provide fully transparent information to help facilitate trade (exporters and importers) of seafood products worldwide and provide information to inform trade liberalisation agreements (Valdimarsson et al, 2004; Ababouch, 2006).

Australian prawns associated with cadmium notifications are believed to be of wild capture origin (no published information exists on levels of cadmium in Australian farmed prawn species). Aquaculture production of finfish such as Southern Bluefin Tuna (*Thunnus maccoyii*) (SBT) has been shown to produce SBT with lower levels of contaminants such as mercury than wild counterpart fish (Padula et al, 2008). No information is available at present to suggest if Australian aquaculture prawns would have lower levels of cadmium than wild

Australian caught prawns. Several prawn species are produced in Australia by aquaculture at different production sites using feeds produced by multiple feed millers. Further research would be necessary to investigate if levels of cadmium in dietary inputs explain levels of cadmium found in harvested product. At least 12 wild caught prawn species are commercially fished in Australia (Kailola *et al*., 1993). These species are: Black or Giant Tiger Prawn (*Penaeus monodon*), Eastern King Prawn (*Penaeus plebejus*), Red Spot King Prawn (*Penaeus longistylus*), Western King Prawn (*Penaeus latisulcatus*), White Banana Prawn (*Penaeus merguiensis*), Red-legged Banana Prawn (*Penaeus indicus*), Brown Tiger Prawn (*Penaeus esculentus*), Grooved Tiger Prawn (*Penaeus semisulcatus*), School Prawn (*Metapenaeus macleayi*), Blue Endeavour Prawn (*Metapenaeus endeavouri*), Red Endeavour Prawn (*Metapenaeus ensis*) and the Greasyback Prawn (*Metapenaeus bennettae*) (Biosecurity Australia, 2009). An Australian Fish Names Standard was introduced in 2007; its use for prawns is not widespread in international markets in part due to local marketing nomenclature for crustaceans (Seafood Services Australia Limited, 2007). This makes identification of prawn species the subject of international market notification more difficult. For example the King Prawn (*Melicortus plebejus*) reported by Greek authorities for cadmium has no entry in the Australian Fish Names Standard and cannot be identified further (Table 3).

Similar to Australian exported prawns to the EU, prawns exported from Indonesia have also come under scrutiny by the European Commisison; in 2006 a legislative approach was taken requiring testing

at port of entry of all Indonesian prawn products for cadmium and other metallic elements (European Commission, 2006).

Of 5,960 port of entry tests conducted over 14,313 consignments of d seafood imported into Australia over 2008, only 150 (2.5%) were found to be non compliant (Moir, 2009). Non compliant seafood products was reported from Sri Lanka, Indonesia, Thailand, Fiji, Italy, Japan, South Korea, Burma, Norway, South Africa, China, Vietnam, Hong Kong, Taiwan, New Zealand, Denmark, Russia, United States, Malaysia and the Philippines. Principal causes of notifications were histamine: 24% (n=36), veterinary medicines 19% (n=29), sulphur dioxide 1% (n=1), microbiological 43% (n=65) and composition 13% (n=19) (Moir, 2009). Overall seafood products account for 14.9% of all imported food testing conducted by Australian authorities (Moir, 2009).

It is speculated that the reasons for differing percentage causes of detentions and rejections for seafood entering Australia against Australian exported Australian seafood may be attributable to several factors including: frequency of testing, number of shipments inspected, number of shipments entering Australia under risk or random surveillance category, use of tariff codes to attract or deflect inspection attention, reliance on exporting countries' export attestation on conformity with Australian standards and laboratory capability (Ababouch, 2005; Moir, 2009; Feazey, 2011). Australian seafood may also be produced under more regulated conditions than other countries such as through official controls on veterinary medicine administration (Australian veterinary association, 2005). Regulatory controls in other

countries may not be as transparent as in Australia. Some countries have introduced Hazard Analysis Critical Control Point (HACCP) programs within their domestic seafood industry (Ababouch, 2006; Isshiki, 2008; Noomhorm and Ahmad, 2008; Maita, 2002; Miyagawa, 2002; Takatori, 2002).

Overall, Australian seafood products have been involved in a very small number of international notifications for breaches of importing countries' food safety standards. Of the cases reported in the present study, 70 % have been due to cadmium in crustaceans. More recently there has been a shift in the causes of detentions and rejections of Australian seafood away from cadmium to other causes such as chemical contaminants (e.g. crystal violet) or natural toxins (e.g. histamine). Large practical differences can be seen in regulatory standards such as for the preservative sulphur dioxide applied by Japanese port of entry authorities against those applied at point of export by Australian authorities (Table 1). Notifications should be interpreted with caution; factors such as frequency of testing, accessibility of non-disclosed notifications, laboratory capability and number of shipments need to be qualified when assessing potential food safety hazards in Australian seafood based purely on international market reports.

Acknowledgements

Mr Simon Liu (Seafood Services Australia) is thanked for translation of Chinese language documents. Ms Rose Flint of the South Australian Research and Development Institute is thanked for technical assistance

with data handling. We would also like to thank Dr Andreas Kiermeier of the South Australian Research and Development Institute for reviewing the manuscript and providing helpful comments.

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CHAPTER EIGHT

General Discussion

Baseline data for a range of residues of public health and market access importance generated as part of this thesis show that Australian wild capture and aquaculture finfish species examined contain very low or negligible levels of these contaminants. Key achievements from this research have led to the removal of Southern Bluefin Tuna (SBT) from the Food Standards Australia New Zealand mercury advisory statement in 2004 (Food Standards Australia New Zealand, 2004) (Chapter five). In 2005 a decision by the Japanese Government to revise their national public health advisory statement saw a differentiation between SBT and other bluefin tuna species (Ministry of Health, Labour and Welfare, 2005) (Chapter five). With Japan being the dominant export market for Australian SBT, the Australian Government in 2004 adopted Japanese port of entry sampling techniques to allow equivalent data to be generated as part of the National Dioxins Program for Australian aquaculture-produced SBT (Department of Agriculture, Fisheries and Forestry, 2004) (Chapters two and three). Farmed Yellowtail Kingfish (YTKF) and Mulloway were unknown in the domestic and some international markets until recently. Following the generation of a baseline residue profile, the industry now participates in an annual formal residue control program giving them access to the European Union (EU) (Feazey, 2011) (Chapter four). Australian canned tuna products investigated for mercury content satisfied United States Government criteria to support a front of pack "low in mercury" label which led to a series of supplier contracts with United States supermarket chains (Austin, 2006) (Chapter six). Work on wild SBT has resulted in the Australian food regulator reviewing its mercury advisory statement again in 2011; emphasis has now been

changed from discouraging fish consumption of numerous listed species to providing information in simple accessible language for particular population groups (Food Standards Australia New Zealand, 2011) (Chapter five). Further work is being undertaken in relation to developing national animal feed standards to set permissible levels for the presence of pollutants in animal feeds; thereby preventing violative ingredients from entering the food chain (Department of Agriculture, Fisheries and Forestry, 2010). Trade detentions and rejections of Australian seafood in international trade have principally been due to cadmium in Australian crustaceans (Chapter seven).

Dioxins and PCBs

Chapters two, three and four provide individual case studies examining the persistent chlorinated organic pollutants dioxins and PCBs in three species of Australian marine finfish. These investigations found overall very low levels of dioxins and polychlorinated biphenyls (PCBs). Results reported for farmed SBT in this thesis are consistent with those previously published (Phua et al, 2008). The types of dioxins and PCBs present in the three marine finfish species suggested emission sources such as bushfires were the probable origin of these residues. Australia and New Zealand has in the past manufactured and used some dioxin contaminated building treatment products such as trichlorophenol, a once commonly but no longer used wood preservative to prevent termite infestation of house structures (Collins, 2009).

Toxic Equivalent Factor (TEF) values published in 2006 have lessened the contribution of the dioxin-like PCBs to the total Toxic Equivalent (TEQ) values (Van den Berg et al, 1998; Van den Berg et al 2006). These TEF values are key regulatory tools to allow description and comparison of data internationally for compliance purposes. This will create difficulties in comparisons between current and historical data. The European Union has not adopted the 2005 TEF values but has chosen to retain the 1998 values when the new Maximum Level (ML) for the sum of the dioxins and the dioxin-like PCBs was announced (Van den Berg et al, 1998; Van den Berg et al, 2006; European Commission, 2006). The United States has adopted the use of the 2005 TEF values (Environment Protection Agency, 2010). The TEF values are consensus figures, they do not necessarily take into consideration interspecies differences or aquatic food producing animal production differences (aquaculture vs. wild capture). They reflect toxicity acting through the aryl hydrocarbon receptor and not by any other mechanisms. It has been almost 30 years since the first discussion of a TEF type approach for reporting of dioxin data (Environment Protection Agency, 2010). In the case of SBT the majority of the TEQ value is contributed by PCB 126, hence the impacts of these changes in this case are very small.

Mercury

Chapters five and six described case studies examining total mercury content in two wild tuna species. Dietary modelling conducted as part of this thesis on a variety of finfish species provides updated information on consumer dietary exposure assessments from

consumption of these products. Chapter six demonstrates Australian canned tuna products produced with SJT contain very low levels of mercury and can be consumed by all Australian consumers. The Australian and subsequent Japanese public mercury advisory statement revision for SBT is an example of the benefits of open scientific communication (Ministry of Health, Labour and Welfare, 2004; Ministry of Health, Labour and Welfare, 2005).

The mean concentration of total mercury in wild Australian SBT was 0.36 mg/kg (range 0.31-0.41) while in farmed SBT it was 0.31 (range 0.18-0.45) (Chapter three). Total mercury content of wild Atlantic Bluefin Tuna (ABT) (*Thunnus thynnus*) (n=7) from the Ionian Sea has been reported as ranging between 0.13-0.35 mg/kg (Storelli et al., 2005), while farmed ABT from the Adriatic Sea had a maximum total mercury concentration of 1.8 mg/kg (Srebocan, 2007). The reason why different species of tuna have higher or lower concentrations of mercury present is related to several factors including each species' trophic position in the marine food chain and the contamination level of the diet which maybe temporally and spatially variable (Dietz et al., 2000).

Diet is also a factor influencing mercury content of farmed tuna species. Japanese researchers have been able to demonstrate reductions in levels of mercury in farmed Pacific Bluefin Tuna (*Thunnus orientalis*) by feeding low mercury content baitfish including Spotted Chub Mackerel (*Scomber australasicus*) (Nakao et al, 2009). Other factors can affect mercury bioaccumulation including water temperature and feed intake in turn (Ando, 2010; Ando et al 2011). Water temperature may influence

lipid deposition and growth, hence indirectly affecting mercury content. The Japanese studies occurred in water temperatures 21-29°C, with a marked decline in feed intake occurring in December and January (winter). An apparent reduction in mercury concentration in the experimental tuna could be seen in the months of July and August (summer) as water temperatures rose, but only at the Oshima Experimental Station, Wakayama, Japan. No effect was seen at the Amami Experimental Station, Kagoshima, Japan. Peak mercury concentrations in the farmed tuna coincided with coldest water temperature readings (Ando, 2010). The exact reaons for this observation are not known but it is speculated that fat content of these fish may have been lower at this time of year in response to reduced feed intake.

Pesticides

All finfish regardless of species showed no residues of any organochlorine or organophosphate pesticide compounds. This may be due in part to action by Australian authorities in cancelling registration of environmentally persistent pesticides such as DDT in the mid 1980s (Connell et al, 2002). Such regulatory controls may take years or decades to be effective in practice due to the persistent nature of some of these compounds in the marine environment. However, not all developing countries have taken this approach due to immediate domestic needs to alleviate crop infestations or control public health nuisance insects for malaria management. Different climatic conditions in some countries such as monsoonal rain may rapidly mobilise terrestrially applied crop protection chemicals into rivers and marine

waters. Some tuna species such as Skipjack Tuna (SJT) (*Katsuwonus pelamis*), due to their migratory lifecycle may accumulate pollutants such as organochlorine pesticides from prey items consumed while transiting polluted areas, in effect acting as biological indicators of those areas (Ueno et al, 2003). No pesticides were found in farmed SBT, YTKF or Mulloway tested as part of this thesis (Chapters three and four).

Veterinary medicines

No detectable residues of any antimicrobial compounds were found in any farmed finfish species covered in this thesis (Chapters three and four). Testing did not include skin; fish skin may contain a number of organic pollutants, veterinary medicines and potentially metallic elements such as cadmium and lead (Abe and Fuchino, 2001). Fish bone may also selectively accumulate veterinary medicines such as tetracyclines. The selection of which portion to test for any compound needs to be guided by information on the distribution of that compound in fish; if testing is for compliance purposes it may need to also consider other issues like testing of non-edible tissue to determine presence of prohibited compounds. Different culinary traditions in each market mean that testing of selective portions (skin off fillet) may not always provide information on potential risks in exported produce. On the other hand, practical considerations may mean it is impractical or impossible to process whole large fish for testing particularly if the fish are of high economic value.

In Australia, veterinary surgeons are registered separately by each of the State or Territory Government; prescribing privileges (to food producing animals) are available only to veterinary surgeons registered in that particular State or Territory (Australian Veterinary Association, 2005). Along with these prescribing privileges comes legal authority to administer unregistered products or to prescribe off label (that is to give a registered product to an animal that it is not registered to be administered to and or at a dose or duration contrary to the label advice) (Australian Veterinary Association, 2005). For each of these options, a with-holding period generally must be observed before the animal can be harvested for food consumption. Australian veterinary surgeons must have full knowledge of the market destination requirements of registered and off label prescribed veterinary medicines to set a withholding period or an export slaughter interval (Australian Veterinary Association, 2005). Off label prescribing allows use of a registered drug or veterinary chemical in a manner outside the range of uses permitted by the approved label directions, including species of animal, dosage, treatment interval etc. (but not contrary to a specific label restraint). Veterinary surgeons may exercise professional judgement in the off label use or supply medicines to treat animals. This gives veterinarians access to potentially beneficial drugs which may be registered for human use or which have limited registration for veterinary use (Australian Veterinary Association, 2005).

Longer term, access to a wider range of registered veterinary medicine products for use by the Australian aquaculture industry may occur if supporting field studies are undertaken to verify efficacy, safety and

withholding periods for these compounds. In countries such as Japan, registered anthelmintic medicines such as praziquantel may be administered to farmed fish (National Veterinary Assay Laboratory, 2011).

Feeds

Australian aquaculture feeds such as those fed to YTKF and Mulloway are subject to in-house residue testing by aquaculture feed millers (Rose, 2009). Testing by commercial feed suppliers assists the Australian aquaculture industry by identifying high risk feed components before they are milled into finished products.

Feeding studies performed on farmed Atlantic Salmon (*Salmo salar*), showed that some dietary contaminants such as the dioxin-like PCBs are more efficiently accumulated than PCDD/Fs (Lundebye et al, 2004). Salmon fed an experimental diet for 7 months accumulated the dioxin-like PCBs in proportion to the contamination level in the feed. However, the PCDD/F and dioxin-like PCB concentration was not measured in the feed fed to the salmon prior to the experiment. The retention rate (from feed) for the dioxin-like PCBs was 84% and for the PCDD/Fs it was almost half at 49%. The TEQ was dominated by the contribution of the dioxin-like PCBs providing up to 78% of the total value. This suggests greater attention should be paid to the concentration of dioxin-like PCBs in feeds and feed ingredients than to the total overall TEQ concentration.

Australian regulatory controls

Australian seafood is subject to a number of regulatory controls, these include licenses to catch or produce species, catch limits, food safety programs on board of the fishing boats and in processing establishment and international free trade agreements.

Aquaculture products bound for the EU must participate in an annual residue control program (The Council of the European Union, 1996). Australian wild capture seafood products also voluntarily participate in an annual residue testing program to facilitate market access (Feazey, 2011). These annual testing programs include SBT, YTKF and Mulloway. These programs provide industry with official internationally recognised assurances need to maintain market access (Thilmany and Barrett 1997; Antle, 1999).

Trade implications

Chapter seven described the causes of rejections and detentions of Australian seafood in international trade. The largest issue facing Australian seafood exporters on a numerical basis has been cadmium in prawn species entering European markets. Trade reports should be used with caution without access to qualifying information on frequency of testing, non-disclosed notifications, number of shipments inspected, sampling information, laboratory methodology and importing country's standards technical basis (Chapter seven).

Port of entry inspection programs are guided by domestic requirements of the importing country may see testing undertaken in a manner

different to that undertaken for conformity assessment by the exporting competent authority (Chapter seven). Of the four species of marine finfish covered in this thesis, none were detained or rejected for presence of residues or contaminants investigated in this thesis. The single case of YTKF found with crystal violet has led to the inclusion of this dye in the standard test panel that all Australian aquaculture products are tested for. This further supports the results presented in this thesis, that the chemical residues in SBT, YTKF or Mulloway are either low or below laboratory detection limits. In contrast, internationally the causes of detentions and rejections are highly variable depending on species, country of export and country of import (Ababouch et al, 2005; Ababouch, 2006; Valdimarsson et al, 2004).

The development of an online database containing each country's food regulatory standards, sampling methodology, laboratory methodology and related information would assist all parties by creating complete transparency of this information. Organisations like the World Trade Organisation (WTO) already provide formal notifications of proposed changes, but do not keep information on current implemented standards in any accessible format. In contrast, for animal diseases globally, the Office International des Epizooties (OIE) has been in operation since 1924 (Office International des Epizooties, 2011). It maintains an online repository of international animal health standards by country (including diagnostic manuals for notifiable pathogens and reference documents) with free access in English, French and Spanish.

The emergence of private purchasing standards by international buyers of Australian seafood may see additional standards or stricter interpretation of existing port of entry requirements applied to Australian products as a price negotiation tool to restrict market access (Henson and Reardon, 2005).

Some residues such as naturally occurring metalloids like selenium in fish may be potentially beneficial to consumers (Mozaffarian and Rimm, 2006; Santerre, 2010). Selenium is an important mineral of public health significance and has a role in mercury harm mitigation in the body (Potter and Matrone, 1974; Ohi et al, 1976). Access to reliable information on minerals and other nutritional components of public health significance may assist to shift consumer negative sentiments about seafood safety (Smith and Riethmuller, 1999; Cohen et al, 2005; Rasco, 2010). Reliable information on these key public health nutrients can provide very persuasive inputs into benefit:risk assessments and risk assessment studies.

Consumers when confronted with inconsistent naming of tuna species combined with non-disclosure of tuna canning species on product packaging may not be able to select low mercury content canned tuna products to meet their individual needs (Chapter five). Combined with differences in exposure assessment methods used by Japan and Australia through use of fixed consumers' body weight values may provide a higher degree of consumer protection than intended (Chapters four and five). Accessibility of this information to consumers

is limited in the retail environment to inform purchasing decision (Chapters five and six).

Sampling design limitations

Due to a number of factors the design of the sample collection programs in Chapters two, three and four was not optimised. Fish are subject to large natural variations in size and population homogeneity; statistical power calculations need to be informed by first hand experimental experience of fish variability (Ling and Cotter, 2003). Fish used in research described in this thesis were commercially valuable and some of the species controlled under international quota arrangements; therefore access to high sample numbers for destructive testing was not possible. The lack of experimental approach was due to a range of factors including, available research funding and availability of a suitable controlled environment facility which could support replicated studies on large pelagic fish such as SBT. While experimental approach could provide a lot of additional information, it was outside of the scope of this thesis.

Laboratory analyses for dioxins and PCBs are costly to undertake. Alternative biological methods would not have provided the congener profile information thereby limiting useful interpretation of such analytical results. Running all samples in duplicate would have provided greater confidence in results reported, but the costs were prohibitive. Sample collection strategies were undertaken using a standardised protocol maximising comparability of data (Thompson and Ramsey, 1995). Published experimental work was used to interpret

results whenever possible. Industry and external funding were used to maximise the sample collection strategy within the financial constraints of the thesis.

Emerging threats

In early 2011 an earthquake struck Japan damaging a nuclear power plant (Japan Cabinet Office, 2011). This has led to low level radionuclide contamination of seafood caught in the immediate area (Ministry of Health, Labour and Welfare, 2011a; Ministry of Health, Labour and Welfare. 2011b; Food Safety Commission, 2011). Potential food safety concerns have seen additional testing and standards applied to imported foods by Australian and international port of entry authorities (Agri-Food and Veterinary Authority of Singapore, 2011; Food Safety and Standards Authority of India, 2011; Food and Environmental Hygiene Department, 2011; Department of Agriculture, Fisheries and Forestry, 2011). It is sometimes difficult for the public to discriminate between a measurable level of a radionuclide contaminant and a real public health threat; they may not feel reassured by knowledge of the presence of trace amounts of these radionuclides or other contaminants in retail products they are purchasing (Wilcock et al, 2004). Radionuclides vary in their toxicity and stability. Some such as iodine isotopes are relatively short-lived, while others such as caesium and plutonium are extremely long-lived in the natural environment (Kryshev et al, 1993).

Recommendations for future work

Regulatory issues

1. The Australian aquaculture industry needs to consider establishing a code of practice for aquaculture feed prior to the introduction of national animal feed standards by the Australian Government. This code of practice would deal with species specific issues of feed additives, residue and contaminant standards for manufactured and baitfish diets used by Australian aquaculture producers. If recognised by vendor quality management standards set by Australian supermarket chains this would create an incentive for its creation.
2. National residue and contaminant testing programs should be consolidated e.g. EU residue control program to include wild and aquaculture products managed under a single program. Feed testing should be included in such a model.
3. An online database containing each country's food/feed regulatory standards, sampling methodology, laboratory methodology and related information should be developed to support industry development. Access to this information would allow transparent comparison of each country's standards to facilitate trade. Other database models already exist for animal diseases (Office International des Epizooties, 2011).
4. A review should be undertaken of current access arrangements to veterinary medicines and agriculture chemicals for use in food producing aquatic animals. This should occur in parallel with the current review of the functions of the Australian competent

authority, Australian Pesticides and Veterinary Medicines Authority.

5. A literature review should be undertaken to identify environmental effects of aquaculture publications to respond to adverse public scrutiny of the Australian aquaculture and wild capture seafood industry.
6. Consideration should be given to development of academic curriculum for graduate and post graduate student programs in the field of seafood product integrity. This may need to draw on international teaching expertise from key markets such as Japan.
7. In-country visits to major markets should be undertaken to maintain current local networks within port of entry laboratories, food regulatory authorities and related research institutions, thus providing a transfer of knowledge portal.
8. A scoping study should be undertaken to identify end users and beneficiaries of Good Laboratory Practice (GLP) capability. GLP capability could be established within a contract research provider to undertake veterinary medicine and agricultural chemical trials to support Australian registration of veterinary medicines and agricultural chemicals in food-producing aquatic animal species.
9. A scoping study should be undertaken to identify potential technology aids to communicate food safety and public health attributes of purchased seafood products as well as identification of end consumers. Mobile phone scanning technology available in countries such as Japan may provide a model for a consumer accessible service.

10. The Australian Fish Names Standard should be extended to include canned fish products to inform consumer purchasing decisions and thereby providing guidance to consumers with particular health needs.

Research issues

1. Ongoing research is required to characterise residues and contaminants including dioxins, PCBs, veterinary medicines, agricultural chemicals, metallic elements and newly emerging persistent organic pollutants (e.g. brominated flame retardants and fluorinated compounds) in Australian wild and farmed aquatic animal products to support risk assessments.
2. Nutritional compositional profiles need to be generated for Australian seafood products to better inform Australian and international consumers on the public health benefits of Australian seafood consumption and to facilitate benefit vs. risk assessments.
3. Information on Australian consumption of seafood products should be collected which includes detailed demographic breakdown, species, wild capture or aquaculture and percentage seafood ingredients if value added products consumed. Additional information is also required on Australian consumers' body weight with detailed demographic breakdown to inform dietary exposure assessments.
4. A through chain risk profile should be undertaken for select priority aquaculture and wild capture species of international trade importance. This risk profile should include

microbiological hazards in addition to chemical hazards. Example studies in terrestrial food animals should be reviewed to identify key issues of interest to food producing aquatic animals.

5. The effect of cooking (including canning) on levels of residues and contaminants in wild and farmed Australian fish needs to be investigated.
6. A national atlas of seafood contamination risk profiles (by species and geographic site) to inform industry market access choices should be developed to guide aquaculture development and fisheries catch site planning.
7. The potential for inclusion of DNA information within the Australian Fish Names Standard to protect provenance of Australian seafood species should be scoped.
8. Experimental evaluation of factors affecting residues and contaminants was not undertaken due to the lack of experimental facility for species such as SBT, YTKF or Mulloway. Establishment of land-based controlled environment replicated tank systems would provide the ability to conduct controlled toxicant exposure and extended grow-out simulation studies. Toxicant exposure studies could be undertaken to determine clearance times of feed contaminants such as pesticides, feed additives (preservatives etc), PCDD/Fs and PCBs. This would help to provide data for national animal feed standards currently in preparation. Investigation of surrogate species (species such as YTKF) in lieu of using SBT to identify effects of climate change on accumulation and depuration of

contaminants should be undertaken. Anthelmintic treatments could be trialled under these controlled conditions. Such facilities if GLP accredited could be used to undertake veterinary medicine trials to accurately establish withholding times at various temperatures and under different dose frequency regimes.

9. Alternative experimental approaches such as use of cell lines could be used to investigate other compounds that do not have dioxin-like effects (that is they do not act on the Aryl hydrocarbon receptor). Cell lines could be used to undertake high replication studies on new veterinary medicine products to demonstrate in vitro toxicity without risking valuable fish or damaging market access. These cell line models have not been demonstrated successfully to date; there are no cell lines available for *Thunnus* or *Seriola* species (pers. comm. Dr Mark Crane, Commonwealth Scientific Industrial Research Organisation).
10. A pilot study should be undertaken to investigate the feasibility of determining carbon content of Australian traded seafood products to inform climate change risk assessments.
11. Risk assessment of natural contaminants e.g. pyrrolizidine alkaloids as fish oil substitutes are evaluated for potential inclusion in aquaculture feed formulations.

Conclusion

This thesis has improved understanding of baseline levels of residues and contaminants of significance to public health and market access in Australian finfish products. Australian finfish species investigated in this thesis are safe to eat (Chapters two, three, four, five, six and seven). Levels of detected residues and contaminants are all well within Australian and international regulatory standards. Thesis outputs have helped to reshape public policies on contaminants such as mercury through provision of new data to inform exposure assessments and risk assessment both within Australia and internationally through modification of public mercury advisory statements in Japan and Australia. Economic outcomes have been measured and will continue to accrue as industry adopts different aspects of the individual studies' findings. Current work is now addressing the issue of regulatory standards for residues and contaminants in aquaculture feeds (Department of Agriculture, Fisheries and Forestry, 2010). This thesis has provided information on residues such as PCDD/PCDFs and PCBs that may potentially accumulate from dietary sources. Access to veterinary medicines and agricultural chemicals is improving with several pilot aquaculture trials underway for several anthelmintic and antifoulant compounds. Industry's greatest challenge is to meet all of these food regulatory needs in a changing world, where there is little consistency between countries and only partial incomplete shifting knowledge available of the workings of these markets.

"All scientific work is incomplete - whether it be observational or experimental. All scientific work is liable to be upset or modified by advancing knowledge. That does not confer upon us a freedom to ignore the knowledge we already have or postpone the action that it appears to demand at a given time." Sir Austin Bradford-Hill (Bradford-Hill, 1965).

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APPENDICES

APPENDIX ONE

Polychlorinated biphenyl nomenclature

Table 1. Summary of polychlorinated biphenyl (PCB) congener nomenclature (United States Environment Protection Agency, 2011).

PCB Homolog	Number of chlorine substituents	Number of PCB congeners
Monochlorobiphenyl	1	3
Dichlorobiphenyl	2	12
Trichlorobiphenyl	3	24
Tetrachlorobiphenyl	4	42
Pentachlorobiphenyl	5	46
Hexachlorobiphenyl	6	42
Heptachlorobiphenyl	7	24
Octachlorobiphenyl	8	12
Nonachlorobiphenyl	9	3
Decachlorobiphenyl	10	1

Reference

United States Environment Protection Agency. 2011. Polychlorinated biphenyls (PCBs). http://www.epa.gov/epawaste/hazard/tsd/pcbs/index.htm Last accessed 13 October 2011.

APPENDIX TWO

Polychlorinated biphenyl trading names

Table 1. Summary of polychlorinated biphenyl (PCB) trade names manufactured worldwide (United States Environment Protection Agency, 2011).

PCB Trade Names		
Aceclor	Diaclor	PCB
Adkarel	Dicolor	PCB's
ALC	Diconal	PCBs
Apirolio	Diphenyl, chlorinated	Pheaoclor
Apirorlio	DK	Phenochlor
Arochlor	Duconal	Phenoclor
Arochlors	Dykanol	Plastivar
Aroclor	Educarel	Polychlorinated biphenyl
Aroclors	EEC-18	Polychlorinated biphenyls
Arubren	Elaol	Polychlorinated diphenyl
Asbestol	Electrophenyl	Polychlorinated diphenyls
ASK	Elemex	Polychlorobiphenyl
Askael	Elinol	Polychlorodiphenyl
Askarel	Eucarel	Prodelec
Auxol	Fenchlor	Pydraul
Bakola	Fenclor	Pyraclor
Biphenyl, chlorinated	Fenocloro	Pyralene
Chlophen	Gilotherm	Pyranol
Chloretol	Hydol	PyroclorPyronol
Chlorextol	Hyrol	Saf-T-Kuhl
Chlorinated biphenyl	Hyvol	Saf-T-Kohl
Chlorinated diphenyl	Inclor	Santosol
Chlorinol	Inerteen	Santotherm
Chlorobiphenyl	Inertenn	Santothern

PCB Trade Names		
Chlorodiphenyl	Kanechlor	Santovac
Chlorphen	Kaneclor	Solvol
Chorextol	Kennechlor	Sorol
Chorinol	Kenneclor	Soval
Clophen	Leromoll	Sovol
Clophenharz	Magvar	Sovtol
Cloresil	MCS 1489	Terphenychlore
Clorinal	Montar	Therminal
Clorphen	Nepolin	Therminol
Decachlorodiphenyl	No-Flamol	Turbinol
Delor	NoFlamol	
Delorene	Non-Flamol	
	Olex-sf-d	
	Orophene	

Reference

United States Environment Protection Agency. 2011. Polychlorinated biphenyls (PCBs). http://www.epa.gov/epawaste/hazard/tsd/pcbs/index.htm Last accessed 13 October 2011.

APPENDIX THREE

International Union of Pure and Applied Chemists polychlorinated biphenyl nomenclature

Table 1. Summary of International Union of Pure and Applied Chemists (IUPAC) nomenclature for polychlorinated biphenyls (PCBs) (United States Environment Protection Agency, 2011).

PCB congener number	IUPAC scientific name
1	2-Chlorobiphenyl
2	3-Chlorobiphenyl
3	4-Chlorobiphenyl
4	2,2'-Dichlorobiphenyl
5	2,3-Dichlorobiphenyl
6	2,3'-Dichlorobiphenyl
7	2,4-Dichlorobiphenyl
8	2,4'-Dichlorobiphenyl
9	2,5-Dichlorobiphenyl
10	2,6-Dichlorobiphenyl
11	3,3'-Dichlorobiphenyl
12	3,4-Dichlorobiphenyl
13	3,4'-Dichlorobiphenyl
14	3,5-Dichlorobiphenyl
15	4,4'-Dichlorobiphenyl
16	2,2',3-Trichlorobiphenyl
17	2,2',4-Trichlorobiphenyl
18	2,2',5-Trichlorobiphenyl
19	2,2',6-Trichlorobiphenyl
20	2,3,3'-Trichlorobiphenyl
21	2,3,4-Trichlorobiphenyl
22	2,3,4'-Trichlorobiphenyl

PCB congener number	IUPAC scientific name
23	2,3,5-Trichlorobiphenyl
24	2,3,6-Trichlorobiphenyl
25	2,3',4-Trichlorobiphenyl
26	2,3',5-Trichlorobiphenyl
27	2,3',6-Trichlorobiphenyl
28	2,4,4'-Trichlorobiphenyl
29	2,4,5-Trichlorobiphenyl
30	2,4,6-Trichlorobiphenyl
31	2,4',5-Trichlorobiphenyl
32	2,4',6-Trichlorobiphenyl
33	2,3',4'-Trichlorobiphenyl
34	2,3',5'-Trichlorobiphenyl
35	3,3',4-Trichlorobiphenyl
36	3,3',5-Trichlorobiphenyl
37	3,4,4'-Trichlorobiphenyl
38	3,4,5-Trichlorobiphenyl
39	3,4',5-Trichlorobiphenyl
40	2,2',3,3'-Tetrachlorobiphenyl
41	2,2',3,4-Tetrachlorobiphenyl
42	2,2',3,4'-Tetrachlorobiphenyl
43	2,2',3,5-Tetrachlorobiphenyl
44	2,2',3,5'-Tetrachlorobiphenyl
45	2,2',3,6-Tetrachlorobiphenyl
46	2,2',3,6'-Tetrachlorobiphenyl
47	2,2',4,4'-Tetrachlorobiphenyl
48	2,2',4,5-Tetrachlorobiphenyl
49	2,2',4,5'-Tetrachlorobiphenyl
50	2,2',4,6-Tetrachlorobiphenyl
51	2,2',4,6'-Tetrachlorobiphenyl
52	2,2',5,5'-Tetrachlorobiphenyl

PCB congener number	IUPAC scientific name
53	2,2',5,6'-Tetrachlorobiphenyl
54	2,2',6,6'-Tetrachlorobiphenyl
55	2,3,3',4-Tetrachlorobiphenyl
56	2,3,3',4'-Tetrachlorobiphenyl
57	2,3,3',5-Tetrachlorobiphenyl
58	2,3,3',5'-Tetrachlorobiphenyl
59	2,3,3',6-Tetrachlorobiphenyl
60	2,3,4,4'-Tetrachlorobiphenyl
61	2,3,4,5-Tetrachlorobiphenyl
62	2,3,4,6-Tetrachlorobiphenyl
63	2,3,4',5-Tetrachlorobiphenyl
64	2,3,4',6-Tetrachlorobiphenyl
65	2,3,5,6-Tetrachlorobiphenyl
66	2,3',4,4'-Tetrachlorobiphenyl
67	2,3',4,5-Tetrachlorobiphenyl
68	2,3',4,5'-Tetrachlorobiphenyl
69	2,3',4,6-Tetrachlorobiphenyl
70	2,3',4',5-Tetrachlorobiphenyl
71	2,3',4',6-Tetrachlorobiphenyl
72	2,3',5,5'-Tetrachlorobiphenyl
73	2,3',5',6-Tetrachlorobiphenyl
74	2,4,4',5-Tetrachlorobiphenyl
75	2,4,4',6-Tetrachlorobiphenyl
76	2,3',4',5'-Tetrachlorobiphenyl
77	3,3',4,4'-Tetrachlorobiphenyl
78	3,3',4,5-Tetrachlorobiphenyl
79	3,3',4,5'-Tetrachlorobiphenyl
80	3,3',5,5'-Tetrachlorobiphenyl
81	3,4,4',5-Tetrachlorobiphenyl
82	2,2',3,3',4-Pentachlorobiphenyl

PCB congener number	IUPAC scientific name
83	2,2',3,3',5-Pentachlorobiphenyl
84	2,2',3,3',6-Pentachlorobiphenyl
85	2,2',3,4,4'-Pentachlorobiphenyl
86	2,2',3,4,5-Pentachlorobiphenyl
87	2,2',3,4,5'-Pentachlorobiphenyl
88	2,2',3,4,6-Pentachlorobiphenyl
89	2,2',3,4,6'-Pentachlorobiphenyl
90	2,2',3,4',5-Pentachlorobiphenyl
91	2,2',3,4',6-Pentachlorobiphenyl
92	2,2',3,5,5'-Pentachlorobiphenyl
93	2,2',3,5,6-Pentachlorobiphenyl
94	2,2',3,5,6'-Pentachlorobiphenyl
95	2,2',3,5',6-Pentachlorobiphenyl
96	2,2',3,6,6'-Pentachlorobiphenyl
97	2,2',3,4',5'-Pentachlorobiphenyl
98	2,2',3,4',6'-Pentachlorobiphenyl
99	2,2',4,4',5-Pentachlorobiphenyl
100	2,2',4,4',6-Pentachlorobiphenyl
101	2,2',4,5,5'-Pentachlorobiphenyl
102	2,2',4,5,6'-Pentachlorobiphenyl
103	2,2',4,5',6-Pentachlorobiphenyl
104	2,2',4,6,6'-Pentachlorobiphenyl
105	2,3,3',4,4'-Pentachlorobiphenyl
106	2,3,3',4,5-Pentachlorobiphenyl
107	2,3,3',4',5-Pentachlorobiphenyl
108	2,3,3',4,5'-Pentachlorobiphenyl
109	2,3,3',4,6-Pentachlorobiphenyl
110	2,3,3',4',6-Pentachlorobiphenyl
111	2,3,3',5,5'-Pentachlorobiphenyl
112	2,3,3',5,6-Pentachlorobiphenyl

PCB congener number	IUPAC scientific name
113	2,3,3',5',6-Pentachlorobiphenyl
114	2,3,4,4',5-Pentachlorobiphenyl
115	2,3,4,4',6-Pentachlorobiphenyl
116	2,3,4,5,6-Pentachlorobiphenyl
117	2,3,4',5,6-Pentachlorobiphenyl
118	2,3',4,4',5-Pentachlorobiphenyl
119	2,3',4,4',6-Pentachlorobiphenyl
120	2,3',4,5,5'-Pentachlorobiphenyl
121	2,3',4,5',6-Pentachlorobiphenyl
122	2,3,3',4',5'-Pentachlorobiphenyl
123	2,3',4,4',5'-Pentachlorobiphenyl
124	2,3',4',5,5'-Pentachlorobiphenyl
125	2,3',4',5',6-Pentachlorobiphenyl
126	3,3',4,4',5-Pentachlorobiphenyl
127	3,3',4,5,5'-Pentachlorobiphenyl
128	2,2',3,3',4,4'-Hexachlorobiphenyl
129	2,2',3,3',4,5-Hexachlorobiphenyl
130	2,2',3,3',4,5'-Hexachlorobiphenyl
131	2,2',3,3',4,6-Hexachlorobiphenyl
132	2,2',3,3',4,6'-Hexachlorobiphenyl
133	2,2',3,3',5,5'-Hexachlorobiphenyl
134	2,2',3,3',5,6-Hexachlorobiphenyl
135	2,2',3,3',5,6'-Hexachlorobiphenyl
136	2,2',3,3',6,6'-Hexachlorobiphenyl
137	2,2',3,4,4',5-Hexachlorobiphenyl
138	2,2',3,4,4',5'-Hexachlorobiphenyl
139	2,2',3,4,4',6-Hexachlorobiphenyl
140	2,2',3,4,4',6'-Hexachlorobiphenyl
141	2,2',3,4,5,5'-Hexachlorobiphenyl
142	2,2',3,4,5,6-Hexachlorobiphenyl

PCB congener number	IUPAC scientific name
143	2,2',3,4,5,6'-Hexachlorobiphenyl
144	2,2',3,4,5',6-Hexachlorobiphenyl
145	2,2',3,4,6,6'-Hexachlorobiphenyl
146	2,2',3,4',5,5'-Hexachlorobiphenyl
147	2,2',3,4',5,6-Hexachlorobiphenyl
148	2,2',3,4',5,6'-Hexachlorobiphenyl
149	2,2',3,4',5',6-Hexachlorobiphenyl
150	2,2',3,4',6,6'-Hexachlorobiphenyl
151	2,2',3,5,5',6-Hexachlorobiphenyl
152	2,2',3,5,6,6'-Hexachlorobiphenyl
153	2,2',4,4',5,5'-Hexachlorobiphenyl
154	2,2',4,4',5,6'-Hexachlorobiphenyl
155	2,2',4,4',6,6'-Hexachlorobiphenyl
156	2,3,3',4,4',5-Hexachlorobiphenyl
157	2,3,3',4,4',5'-Hexachlorobiphenyl
158	2,3,3',4,4',6-Hexachlorobiphenyl
159	2,3,3',4,5,5'-Hexachlorobiphenyl
160	2,3,3',4,5,6-Hexachlorobiphenyl
161	2,3,3',4,5',6-Hexachlorobiphenyl
162	2,3,3',4',5,5'-Hexachlorobiphenyl
163	2,3,3',4',5,6-Hexachlorobiphenyl
164	2,3,3',4',5',6-Hexachlorobiphenyl
165	2,3,3',5,5',6-Hexachlorobiphenyl
166	2,3,4,4',5,6-Hexachlorobiphenyl
167	2,3',4,4',5,5'-Hexachlorobiphenyl
168	2,3',4,4',5',6-Hexachlorobiphenyl
169	3,3',4,4',5,5'-Hexachlorobiphenyl
170	2,2',3,3',4,4',5-Heptachlorobiphenyl
171	2,2',3,3',4,4',6-Heptachlorobiphenyl
172	2,2',3,3',4,5,5'-Heptachlorobiphenyl

PCB congener number	IUPAC scientific name
173	2,2',3,3',4,5,6-Heptachlorobiphenyl
174	2,2',3,3',4,5,6'-Heptachlorobiphenyl
175	2,2',3,3',4,5',6-Heptachlorobiphenyl
176	2,2',3,3',4,6,6'-Heptachlorobiphenyl
177	2,2',3,3',4,5',6'-Heptachlorobiphenyl
178	2,2',3,3',5,5',6-Heptachlorobiphenyl
179	2,2',3,3',5,6,6'-Heptachlorobiphenyl
180	2,2',3,4,4',5,5'-Heptachlorobiphenyl
181	2,2',3,4,4',5,6-Heptachlorobiphenyl
182	2,2',3,4,4',5,6'-Heptachlorobiphenyl
183	2,2',3,4,4',5',6-Heptachlorobiphenyl
184	2,2',3,4,4',6,6'-Heptachlorobiphenyl
185	2,2',3,4,5,5',6-Heptachlorobiphenyl
186	2,2',3,4,5,6,6'-Heptachlorobiphenyl
187	2,2',3,4',5,5',6-Heptachlorobiphenyl
188	2,2',3,4',5,6,6'-Heptachlorobiphenyl
189	2,3,3',4,4',5,5'-Heptachlorobiphenyl
190	2,3,3',4,4',5,6-Heptachlorobiphenyl
191	2,3,3',4,4',5',6-Heptachlorobiphenyl
192	2,3,3',4,5,5',6-Heptachlorobiphenyl
193	2,3,3',4',5,5',6-Heptachlorobiphenyl
194	2,2',3,3',4,4',5,5'-Octachlorobiphenyl
195	2,2',3,3',4,4',5,6-Octachlorobiphenyl
196	2,2',3,3',4,4',5,6'-Octachlorobiphenyl
197	2,2',3,3',4,4',6,6'-Octachlorobiphenyl
198	2,2',3,3',4,5,5',6-Octachlorobiphenyl
199	2,2',3,3',4,5,5',6'-Octachlorobiphenyl
200	2,2',3,3',4,5,6,6'-Octachlorobiphenyl
201	2,2',3,3',4,5',6,6'-Octachlorobiphenyl
202	2,2',3,3',5,5',6,6'-Octachlorobiphenyl

PCB congener number	IUPAC scientific name
203	2,2',3,4,4',5,5',6-Octachlorobiphenyl
204	2,2',3,4,4',5,6,6'-Octachlorobiphenyl
205	2,3,3',4,4',5,5',6-Octachlorobiphenyl
206	2,2',3,3',4,4',5,5',6-Nonachlorobiphenyl
207	2,2',3,3',4,4',5,6,6'-Nonachlorobiphenyl
208	2,2',3,3',4,5,5',6,6'-Nonachlorobiphenyl
209	Decachlorobiphenyl

Reference

United States Environment Protection Agency. 2011. Polychlorinated biphenyls (PCBs). http://www.epa.gov/epawaste/hazard/tsd/pcbs/index.htm Last accessed 13 October 2011.

APPENDIX FOUR

World Health Organisation dioxin and dioxin-like PCB Toxic Equivalent Factor values

Table 1. World Health Organisation Toxic Equivalent Factor values and revisions for dioxins an dioxin-like PCBs (Van den Berg et al, 1998; Van den Berg et al, 2006).

Compound	WHO 1998 TEF	WHO 2005 TEF
Chlorinated dibenzo-p-dioxins		
2,3,7,8-TCDD	1	1
1,2,3,7,8-PeCDD	1	1
1,2,3,4,7,8-HxCDD	0.1	0.1
1,2,3,6,7,8-HxCDD	0.1	0.1
1,2,3,7,8,9-HxCDD	0.1	0.1
1,2,3,4,6,7,8-HpCDD	0.01	0.01
OCDD	0.0001	0.0003
Chlorinated dibenzofurans		
2,3,7,8-TCDF	0.1	0.1
1,2,3,7,8-PeCDF	0.05	0.03
2,3,4,7,8-PeCDF	0.5	0.3
1,2,3,4,7,8-HxCDF	0.1	0.1
1,2,3,6,7,8-HxCDF	0.1	0.1
1,2,3,7,8,9-HxCDF	0.1	0.1
2,3,4,6,7,8-HxCDF	0.1	0.1
1,2,3,4,6,7,8-HpCDF	0.01	0.01
1,2,3,4,7,8,9-HpCDF	0.01	0.01
OCDF	0.0001	0.0003
Non-ortho-substitututed PCBs		
PCB 77	0.0001	0.0001
PCB 81	0.0001	0.0003
PCB 126	0.1	0.1

Compound	WHO 1998 TEF	WHO 2005 TEF
PCB 169	0.01	0.03
Mono-ortho-substitututed PCBs		
PCB 105	0.0001	0.00003
PCB 114	0.0005	0.00003
PCB 118	0.0001	0.00003
PCB 123	0.0001	0.00003
PCB 156	0.0005	0.00003
PCB 157	0.0005	0.00003
PCB 167	0.00001	0.00003
PCB 169	0.0001	0.00003

References

Van den Berg, M., Birnbaum, L., Bosveld, A.T.; Brunström, B., Cook, P., Feeley, M., Giesy, J.P., Hanberg, A., Hasegawa, R., Kennedy, S.W., Kubiak, T.O., Larsen, J.C., Van Leeowen, R.F.X., Liem D.A.K., Nolt, C., Peterson, R.E., Poellinger, L., Safe, S., Schrenk, D., Tillitt, D., Tysklind, M., Younes, M., Wærn, Zacharewsk, T., 1998. Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. Envir. Health Persp. 106, 774-792.

Van den Berg, M., Birnbaum, L.S., Denison, M., De Vito, M., Farland, W., Feeley, M., Fiedler, H., Hakansson, H., Hanberg, A., Haws, L., Rose, M., Safe, S., Schrenk, D., Tohyama, C., Tritscher, A., Tuomisto, J., Tysklind, M., Walker, N., Peterson, R.E. 2006. The 2005 World Health Organization Reevaluation of Human and Mammalian Toxic Equivalency Factors for Dioxins and Dioxin-Like Compounds. Tox. Sci. 93, 223-241.

APPENDIX FIVE

Japanese Government sampling protocol for compliance testing of imported bluefin tunas

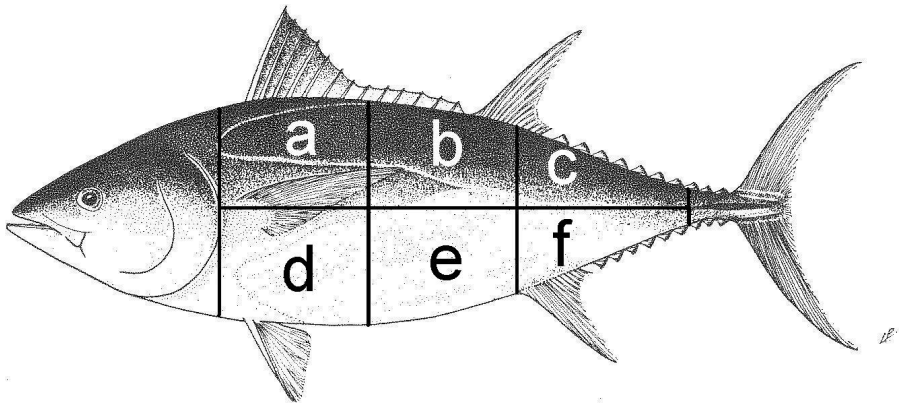

Figure 1. Diagrammatic representation of individual cuts that form the basis of Japanese Government port of entry residue testing programs for imported tunas (pers. comm. Mr Yuichi Nagaka).

Figure 1 is a diagrammatic representation of the Japanese Ministry of Agriculture, Forestry and Fisheries (MAFF) port of entry sample collection protocol for bluefin tunas based on the clockwise collection of portions indicated (a-f) from 10 different bluefin tunas. These 10 individual samples from each of the bluefin tunas are then pooled to form the analytical sample.

Reference

pers. comm. Mr Yuichi Nagaka. 2004. Japanese Ministry of Agriculture, Forestry and Fisheries.

APPENDIX SIX

Overview of Japanese Government food regulatory system

Residue and contaminant standards in Japan are regulated by different arms of the Japanese Government (Anon, 2011a; Anon, 2011b; Anon, 2011c). An overview is given below of how this system functions.

Japanese Government Cabinet Office

- Minister of State for Food Safety

Japanese Food Safety Commission

Functions

- Formed in July 2003 to ally public confidence in Government public health management of the food chain,
- Risk assessment of chemicals of public health and trade significance,
- Risk communication,
- Emergency response,
- Collection and exchange of information with foreign governments, international organisations and other relevant Japanese Ministries,
- Making recommendations to other Japanese Ministries, and
- Responsible directly to the Japanese Prime Minister.

Japanese Ministry of Health, Labour and Welfare

Functions

- Risk management in relation to food sanitation;

- Approval of food additives;
- Formulation of residue and contaminant standards;
- Formulation of standards for food processing and manufacturing;
- Ensuring food safety through monitoring guidance in manufacturing, distribution and retail levels; and
- Implementation of risk communication.

Japanese Ministry of Agriculture, Forestry and Fisheries

Functions

- Risk management in relation to agriculture, forestry and fisheries products (including aquaculture);
- Ensuring safety and regulatory compliance for primary produce;
- Ensuring safety through activities for the improvement of production, distribution and consumption of agriculture, forestry and fisheries products; and
- Implementation of risk communication.

References

Anon, 2011a. Japanese Ministry of Health, Labour and Welfare. www.mhlw.go.jp Last accessed 13 October 2011.

Anon, 2011b. Food Safety Commission. www.fsc.go.jp Last accessed 13 October 2011.

Anon, 2011c. Ministry of Agriculture, Forestry and Fisheries. www.maff.go.jp Last accessed 13 October 2011.

APPENDIX SEVEN

Portion of bluefin tunas to which regulatory standards apply to in international trade

Table 1. Summary of bluefin tuna portion which residue and contaminant standards apply to in international markets.

Compounds	Australia	Japan	Codex Aliementarius Commission	European Union
Dioxins	Not specified	Cross-carcase composite sample formed from 10 individual tunas	Composite bulk sample	The aggregate sample uniting all incremental samples shall be at least 1 kg. In case the lot to be sampled contains small fish (individual fish weighing < 1 kg), the whole fish is taken as incremental sample to form the aggregate sample. In case the resulting aggregate sample weighs more than 3 kg, the incremental samples can consist of the middle part, weighing each at least 100 grams, of the fish forming the aggregate sample. The whole part to which the maximum level is applicable is used for homogenisation of the sample.

Compounds	Australia	Japan	Codex Aliementarius Commission	European Union
				In case the lot to be sampled contains larger fish (individual fish weighing more than 1 kg), the incremental sample consists of the middle part of the fish. Each incremental sample weighs at least 100 grams. In case the lot to be sampled consist of very large fish (e.g. > 6 kg) and taking a piece of the middle part of the fish would result in significant economic damage, taking three incremental samples of at least 350 grams each can be considered sufficient, independently of the size of the lot.
PCBs	The edible content of the food that is ordinarily consumed.	Cross-carcase composite sample formed from 10 individual tunas	Composite bulk sample	The aggregate sample uniting all incremental samples shall be at least 1 kg. In case the lot to be sampled contains small fish (individual fish weighing < 1 kg), the whole fish is taken as incremental sample to form the aggregate sample. In case the resulting aggregate sample

Compounds	Australia	Japan	Codex Aliementarius Commission	European Union
				weighs more than 3 kg, the incremental samples can consist of the middle part, weighing each at least 100 grams, of the fish forming the aggregate sample. The whole part to which the maximum level is applicable is used for homogenisation of the sample. In case the lot to be sampled contains larger fish (individual fish weighing more than 1 kg), the incremental sample consists of the middle part of the fish. Each incremental sample weighs at least 100 grams. In case the lot to be sampled consist of very large fish (e.g. > 6 kg) and taking a piece of the middle part of the fish would result in significant economic damage, taking three incremental samples of at least 350 grams each can be considered sufficient, independently of the size of the lot.

Compounds	**Australia**	**Japan**	**Codex Aliementarius Commission**	**European Union**
Pesticides	Whole commodity including bones and head (in general after removing the digestive tract).	Filleted edible portions from individual fish	Composite bulk sample	Muscle meat
Metals	The edible content of the food that is ordinarily consumed.	Filleted edible portions from individual fish	Composite bulk sample	Muscle meat
Veterinary medicines	Whole commodity including bones and head (in general after removing the digestive tract).	Filleted edible portions from individual fish	Composite bulk sample	Muscle and skin in natural proportions

References

Australia

1. Food Standards Australia New Zealand, Standard 1.4.2, Schedule 4, Foods and classes of foods.

Japan

1. Pers. Comm. Mr Yuichi Nagaka, Japanese Ministry of Agriculture, Forestry and Fisheries.

CODEX Alimentarius Commission

1. CODEX Standard for quick frozen finfish un-eviscerated and eviscerated. CODEX STAN 36-1981, Rev. 1 - 1995.
2. Recommended methods of sampling for the determination of pesticide residues for compliance with MRLs CAC/GL 33-1999.
3. Recommended methods of analysis and sampling. CODEX STAN 234-199.
4. General guidelines on sampling. C/GL 50-2004.
5. FAO/WHO Codex Alimentarius Sampling Plans for Pre-packaged Foods (AQL-6.5) CAC/RM 42-1977.

European Union

1. Commission Directive 2001/22/EC
2. Commission Directive 2004/44/EC
3. Commission Directive 2005/4/EC

APPENDIX EIGHT

Codex Alimentarius Commission committees affecting seafood in international trade

The following Codex Alimentarius Commission committees cover market access and public health issues that may affect Australian seafood products in international trade.

1. Codex Committee on Fish and Fish Products (CCFFP)
2. Codex Committee on Residues of Veterinary Drugs in Foods (CCRVDF)
3. Codex Committee on Contaminants in Foods (CCCF)
4. Codex Committee on Pesticide Residues (CCPR)
5. Codex Committee on Methods of Analysis and Sampling (CCMAS)
6. Codex Committee on Food Hygiene (CCFH)
7. Codex Committee on Food Import and Export Inspection and Certification Systems (CCFIEICS)
8. Codex Committee on Food Additives (CCFA)

Reference

Anon, 2011. CODEX Alimentarius Commission. http://www.codexalimentarius.net/web/archives.jsp?lang=en Last accessed 13 October 2011.